Proteo Vilamoura

Abstract Book



SEProt Sociedad Española de Proteómica

fps French Proteomics Society

PROCURA OMICS PT

Proteo Vilamoura

2nd Joint Meeting of Spanish, French and Portuguese Proteomics Societies

May 11th-13th, 2022, Portugal

More than 30 years after the introduction of the word proteomics, which quickly spread through the scientific literature and beyond, the instrumental development, the development of bioinformatics, the integration with other omics, the sharing of data between researchers, the integration with data coming from other scientific activities, allowed an enormous development of knowledge on living organisms (dead or alive) from viruses to humans but also showed new difficulties and most of all created new challenging opportunities. In all human activities where proteins happen or may come to be proteomics either already exist or will certainly be there in the future. Our proteomics meeting is divided into 4 main sessions referring to: technical and methodological aspects; to different applications; its complementarity with other areas and methodologies (named mixomics); and examples of state-of-the-art works. Reference invited speakers were carefully chosen to share and discuss from the most basic aspects to new subjects that most are unaware of. We also want science to be shared by all who want to do so. Small talks, flash talks and of course posters are programmed so that everyone can share and discuss their work and we all learn together.

It is a pleasure to welcome you to Vilamoura, Algarve to attend ProteoVilamoura 2022. This will be the 2nd Joint Meeting of Spanish, French and Portuguese Proteomics Societies. The meeting was organized to include all aspects of proteomics and related fields focusing on new developments and scientific advances. For this we invited 11 international renowned speakers that will share their work and experiences bringing the opportunity to discuss and share opinions and, at the same time, motivating and inspiring us for novel ideas and projects.

We have chosen this location to give the opportunity, after two years of pandemic, to also relax and enjoy Vilamoura, a low-density population with stunning beaches, a nature trail over five kilometers long, an environmental park with two bird observatories and an award-winning marina.

We look forward to welcoming you to Vilamoura.

Dina Simes
Chairwomen ProteoVilamoura Organizing committee

Dear Colleagues,

It is my great pleasure to welcome you on behalf of PROCURA the Portuguese Proteomics Society, and to invite you to attend the ProteoVilamoura 2022, the 2nd Joint Meeting of Spanish, French and Portuguese Proteomics Societies.

There is no denying that these are ever-changing and unpredictable times, but COVID-19 also show us that nothing can replace the innumerable benefits of meeting people in person and building relationships with them face-to-face. The ProteoVilamoura 2022 conference will allow you to hear and meet those at the forefront of our scientific field and will be a unmissable opportunity to meet face to face with colleagues that share your scientific interests, held in a great and fun location.

This conference goes to the heart of all matters relating to proteomics, and it brings internationally renowned speakers who will share, discuss, debate, and dissect significant new developments and scientific advancements that will impact the future of proteomics and related fields. We also need your work to be shared. This meeting is intended to allow you, irrespective to your personal expertise, to share opinions and to contact expert in all the multiple aspects of proteomics and related fields. The conference program was designed in order to facilitate contacts with the several participants who will guarantee the highest possible scientific level to each session.

Again, it is my honor and privilege, after the success of the previous join meetings with our friends from Spain and France to welcoming you in Algarve, Portugal, and express my hope that together we will make ProteoVilamoura 2022 a memorable event.

Francisco Amado
(President of PROCURA)

It is a pleasure for the French Proteomics Society to co-organize this second edition of a joint meeting with our friends of the Spanish Proteomics Society (SEProt) and of the Portuguese Proteomics Society (Rede ProCura). Previous edition was finally a visioconference due to the COVID19 situation. This second edition should be a face to face event and we can’t wait meeting our dear colleagues once again in person. Our three societies are very active in the field of proteomics and, whatever happens, it should be a great moment of science with a high quality program. We wish you lots of fruitful exchanges and lots of new collaborations.

Sincerely yours,

Franck Vandermoere
(President of FPS)

From the Spanish Society of Proteomics (SEPROT) we want to welcome you to the second edition of the joint meeting with the proteomics societies of France and Portugal.

After these two years marked by teleconferences and travel restrictions, we hope that this meeting will be the starting shot to renew the personal contacts and collaborations that play such an important role in our personal and scientific growth. We are living days full of uncertainty and continuous review of our knowledge where, more than ever in recent times, science is the greatest reason for hope and joy that we have. Let's cultivate that science that we love so much and let's enjoy a productive and dynamic congress, full of new ideas and knowledge. We will see you at ProteoVilamoura 2022 !

Montserrat Carrascal

(President of SEProt)

Organizing Commitee

Dina Simes
(PROCURA, CCMAR-UAlg, Faro, Portugal)
Francisco Amado
(PROCURA, University of Aveiro, Aveiro, Portugal)
Ana Coelho
(PROCURA, ITQB, Oeiras, Portugal)
Carla Viegas
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Scientific Commitee

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(PROCURA, ITQB, Oeiras, Portugal)
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Melisande Blein-Nicolas
(French Proteomics Society, INRA, Gif-sur-Yvette, France)

Proteo Vilamoura

Scientific Programme

Wednesday, May 11th

12.30 Registration
13.30 Welcoming Session
Francisco Amado PROCURA president
Montserrat Carrascal SEProt president (SPS)
Franck Vandermoere FPS president
Dina Simes meeting chair
Nuno Ferreira Bicho vice-rector for Research and Culture at University of Algarve

Proteomics methodology
I CHAIRS: **Montserrat Carrascal / Francisco Amado**

13.50 Symposium 0
Pseudoscience and denialism in times of pandemic
Daniel Closa (IIBB-CSIC-IDIBAPS, Barcelona, Spain)

14.30 Symposium I
Quantitative proteoform profiling of intact human protamines in normozoospermic infertile patients.
Marina Gay (IRB Barcelona, Spain)

15.10 Coffee break sponsored by UNICAM + Poster session I
II CHAIRS: **Franck Vandermoere / Fernando Corrales**

15.40 Symposium II
Proteoforms and their expanding role in biomedical research
Julia Chamot Rooke (Institut Pasteur, Paris, France)

16.20 Symposium III
Tackling cancer mechanisms with spatially resolved proteomics
Isabelle Fournier (PRISM Lab, University of Lille, France)

Oral Communications
CHAIRS: **Deborah Penque / Elsa Lamy**

17.00 Parkinson's disease plasma biomarkers: from translational research to alternative proteomics approaches
Bruno Manadas (CNC-UC, Coimbra, Portugal)

17.15 Proteomics and mass spectrometry imaging study of three-dimensional cell culture of breast tumor
Antonella Raffo-Romero (PRISM Lab, University of Lille, France)

17.30 Sub optimal 15N metabolic labelling in plant: A new way to perform large scale protein turnovers determination using the experimental isotopic distribution.
Willy V. Bienvenut (GQE - IDEEV, Université Paris-Saclay, France)

17.45 Innovative multiplex proteotyping of microbial isolates
Madisson Chabas (CEA, LI2D, Montpellier University, France)

18.00 COMPANY TIME by **Cellenion** - The cellenONE state of the art technology for single cell proteomics & sample preparation

Flash Oral Communications

18.15 Absolute quantitative proteomics using the total protein approach to identify novel clinical immunohistochemical markers in renal neoplasms
Hugo Santos (BIOSCOPE Group, NOVA University of Lisbon, Portugal)

18.20 High-throughput and high-sensitivity biomarker monitoring in body fluid by faims-enhanced fast lc surequant™ is targeted quantitation
Ulrich auf dem Keller (DBB, Technical University of Denmark, Denmark)

18.25 Compared dia-pasef and prm-pasef approaches for the absolute quantification of 500 human plasma proteins in colon cancer plasma samples.
Pierre-Olivier Schmit (Bruker France SAS, Wissembourg, France)

18.30 Metaproteomics analysis of gut microbiota: different processing methods allow the detection of specific phyla
Carmen García-Durán (DMP, FF, Universidad Complutense de Madrid, Spain)

18.35 Undiscovery of regulators of senescence-associated secretory phenotype mediated by small extracellular vesicles
Juan Fafián Labora (GITCMR, FCS, CICA, Universidade da Coruña, Spain)

18.40 S-nitrosoproteomics defines a key mechanism of exercise-induced cardioprotection
Béatrice Alpha-Bazin (LI2D, DMTS/CEA-INRAE, Univ Paris-Saclay, France)

18.45 Protein correlation profiling re-defines the protein composition of extracellular vesicles.
Julia Morales-Sanfrutos (Spanish National Cancer Research Centre - CNIO, Spain)

18.50 Application of 2D DIGE to study the effect of ageing on the myofibrillar sub-proteome of horse meat
Miguel Angel Sentandreu (IATA, CSIC, Spain)

18.55 Relationship between salivary proteome and xerostomia symptoms
Laura Carreira (MED- MIA, University of Évora, Portugal)

19.00 Circulating proteins associated with response and resistance to neoadjuvant chemotherapy in her2-positive breast cancer
María del Pilar Chantada Vázquez (HULA, CHUS, Spain)

19.05 Discussion
19.15 Cocktail reception

Thursday, May 12th

I Proteomics applications and Mixomics
CHAIRS: **Bruno Manadas / Ángel García**

8.30 Symposium IV
Kinase signalling circuits in health and disease
Pedro Beltrão (IMSB, ETH Zurich, Switzerland)

9.10 Symposium V
How proteomics and advanced mass spectrometry are reshaping what we can learn about paintings, objects and cultural heritage
Caroline Tokarski (Inst. of Chemistry, BMNO, University of Bordeaux, France)

9.50 Symposium VI
Evasion of cellular anti-viral defences deciphered by TAILS N-terminomics analysis of SARS CoV-2CLpro substrates in COVID-19
Christopher Overall (CBR, University of British Columbia, Canada)

10.30 Coffee break sponsored by UNICAM + Poster session II

11.00 COMPANY TIME by **Bruker**: Reaching maturity: latest developments in 4D proteomics
Pierre-Olivier Schmit

Oral Communications
CHAIR: **Hugo Osório**

11.30 Proteomic profiling of farmed gilthead seabream skin mucus stress response: a biomarker discovery study
Cláudia Raposo de Magalhães (CCMAR, Universidade do Algarve, Portugal)

11.45 Glycoproteogenomics characterization of the CD44 splicing code in bladder cancer towards precision oncology
José Alexandre Ferreira (CI-IPOP, Portuguese Oncology Institute of Porto, Portugal).

12.00 Quantitative proteomics analysis of aryl-hydrocarbon receptor-interacting protein reveals a novel protein able to regulate tumorigenic and metastatic properties of colorectal cancer cells driving liver metastasis
Guillermo Solís Fernández (MIPD, FS, Belgium /UFIEC, Instituto de Salud Carlos III, Spain)

12.15 Proteomic profiling of ascending aortic VSMC reveals alterations in DNA machinery and cell cycle dysregulation in thoracic aortic aneurysm associated with bicuspid aortic valve
A. Martin-Blazquez (IIS-Fundacion Jimenez Diaz, Immunology, Spain)

12.30 - 14.30 LUNCH BREAK

II Proteomics applications and Mixomics
CHAIR: **Melisande Blein-Nicolas**

14.30 Symposium VII
MaxQuant 2.1.1: Efficient and accurate DDA and DIA-based computational proteomics
Juergen Cox (Max Planck Institute of Biochemistry, Germany)

15.10 Symposium VIII
Antigen discovery for development of personalized cancer immunotherapy
Michal Bassani (Ludwig Institute for Cancer Research Lausanne, Switzerland)

15.50 Coffee break + Poster session III

Oral Communications
CHAIRS: **Carla Viegas / Cristina Ruiz**

16.30 Looking for new plasma biomarkers of Non-Alcoholic Fatty Liver Disease (NAFLD) progression using discovery and targeted proteomics
David Pérez (Univ. Grenoble Alpes, INSERM, CEA, France)

16.45 Identification of novel dysregulated proteins and amyloid-β plaques interactors associated to Alzheimer's disease by proteomics
Ana Montero-Calle (UFIEC, Instituto de Salud Carlos III, Spain)

17.00 Differences and similarities on metabolic response to pH by commensal and pathogenic Staphylococcus epidermidis strains
Elisabete Morais (ITQB, Universidade Nova de Lisboa, Portugal)

17.15 SARS-CoV-2 natural immunity to vaccine-induced immunity: systematic evaluation of humoral response by multipronged functional proteomics approaches
Manuel Fuentes (CIC-IBMCC, USAL-CSIC, IBSAL. Cancer Research Center-IBMCC. University of Salamanca-CSIC, Spain)

17.30 Exploiting glycoengineered simple cells and bioinformatics-assisted glycoproteomics for bladder cancer targets prioritization
Marta Relvas-Santos (Research Center of IPO-Porto and FS-UP, Portugal)

18.00 COMPANY TIME by **Resyn Biosciences**: Hybrid-DIA acquisition strategy combines targeted and discovery proteomics - Jesper Olsen

Flash Oral Communications

18.15 Extensive energy metabolism reprogramming and pathways of neurodegeneration induced by the Inhalation of ultrafine particulate matter revealed by label-free absolute quantitative proteomics
Gonçalo Martins (BIOSCOPE Group, NOVA University of Lisbon, Portugal)

18.20 Proteomics analysis of ΔNp73 effectors identifies proteins associated with lymphan giogenesis, vasculogenesis and metastasis in colorectal cancer
María Garranzo-Asensio (UFIEC, Instituto de Salud Carlos III, Spain)

18.25 Mass-spectrometry based proteomics applied to the conservation of the Iberian lynx (Lynx pardinus)
Guadalupe Gómez-Baena (DBBM, FV-Universidad de Córdoba, Spain)

18.30 Association between calcific aortic valve disease and coronary artery disease: importance of the albumin redox state
Tamara Sastre-Oliva (DVP, Hospital Nacional de Paraplejicos, Spain)

18.35 Tailored proteomic pathway analysis for monitoring of bladder cancer patients
Luís B. Carvalho (BIOSCOPE Group, NOVA University of Lisbon, Portugal)

18.40 Metabolic dyshomeostasis induced by SARS-CoV-2 structural proteins reveals immunological insights into viral olfactory interactions
Mercedes Lachén-Montes (Navarrabiomed, UPNA, and NIHR, Spain)

18.45 Sex-dependent molecular changes in the olfactory tract in Alzheimer's and Parkinson's diseases
Paz Cartas-Cejudo (Navarrabiomed, Universidad Pública de Navarra, Spain)

18.50 Platelet lipidome fingerprint of obese patients: new assistance to characterize platelet dysfunction in obesity
Sara Troitiño (CIMUS, Universidade Santiago de Compostela, and IDIS, Spain)

18.55 Transposable element-derived peptides are preferentially presented on HLA-A3 and HLA-A11 molecules
Yago A. Arribas (Institut Curie, PSL University, France)

19.00 DeST: Deep Semantic Tagger
Francisco M. Couto (LASIGE, Faculdade de Ciências, Universidade de Lisboa, Portugal)

19.05 Discussion

20.00 Conference Dinner (optional, subject to registration and payment)

Friday, May 13th

Advances in Proteomics
CHAIR: **Dina Simes**

8.30 Symposium IX
High-throughput phosphoproteomics to analyze cell signaling networks in health and disease.
Jesper Olsen (University of Copenhagen, Denmark)

9.10 Symposium X
A travel guide to metaproteomics with tips for the study of the most complex samples
Jean Armengaud (CEA, France)

9.50 Coffee break + Poster session IV

Oral Communications
CHAIRS: **Mariette Matondo / Ana Varela Coelho**

10.20 The ghost proteome an hidden vision of the protein landscape
Tristan Cardon (Univ. Lille, PRISM, France).

10.35 The proteome of urinary extracellular vesicles reveals early sub-clinical cardiorenal risk in hypertensive subjects
Miriam Anfaiha-Sanchez (IIS-Fundación Jiménez Díaz Hospital, Spain)

10.50 Does diabetes mellitus change the protein pattern in patients with aortic stenosis?
Nerea Corbacho-Alonso (DVP, Hospital Nacional de Paraplejicos, Spain)

11.05 The histone methyl transferase dot1l regulates chromatin reorganization and mitochondrial activity, and interacts with the pyruvate dehydrogenase complex in differentiating male germ cells
Delphine Pflieger (Univ. Grenoble Alpes, Inserm, CEA, IRIG-BGE, France)

11.20 COMPANY TIME by **Sciex**: Maximizing the performance of the ZenoTOF 7600 system for protein ID and quant

Flash Oral Communications

11.50 Finding biomarkers in thyroid cancer lesions: a mixomics approach
Margarida Coelho (CNC and Cell Biology, FST, University of Coimbra, Portugal)

11.55 Mass spectrometry-based proteomic and metabolomic profiling of serum samples for discovery and validation of tb diagnostic biomarkers
Ana Varela Coelho (ITQB, Universidade Nova de Lisboa, Portugal)

12.00 Spatial proteomic analysis of isogenic metastatic colorectal cancer cells reveals key dysregulated proteins associated with lymph node, liver, and lung metastasis
Rodrigo Barderas (Chronic Dis. Programme, UFIEC, Instituto de Salud Carlos III, Spain)

12.05 Huntacyl: altered dynamics of acylations of histone h3 lysine 27 (h3k27): a mechanism contributing to transcriptional dysregulation in huntington's disease?
Hassan Hijazi (EDyP, University Grenoble Alpes, France)

12.10 Proteomic and phosphoproteomic characterization of molecular mechanisms unlerlying progressive familiar intrahepatic cholestasis type 3
Laura Guerrero (Centro Nacional de Biotecnología CNB-CSIC, Spain)

12.15 Proteomics analysis of gastric cancer patients with diabetes mellitus
Hugo Osório (i3S, Ipatimup and Faculty of Medicine, University of Porto, Portugal)

12.20 Discussion

12.30 Closing Session/Awards

14.00 – 17.00 C-HPP Satellite Workshop on "Accelerating the HPP Grand Challenge", organised by the **Chromosome-centric Human Proteome Project**. See the C-HPP Satellite Workshop agenda here: https://www.hupo.org/News/12690881

Proteo Vilamoura

Poster Sessions

Poster Session I - Wednesday, May 11th - 15.10

I.1 Shotgun proteomics of red blood cells from obstructive sleep apnea patients under positive airway pressure (PAP) treatment
I.2 Investigating the impact of COVID-19 vaccines on the red blood cell immune function by omics-based approaches
I.3 Occupational secondhand smoke exposure - a proteomic analysis
I.4 Endothelial colony forming cells (ECFCs) proteomic response to the serum factors of COVID19 asymptomatic or critical patients
I.5 Swath-ms reveals new insights in the cellular pathophysiology of human heart failure with preserved ejection fraction (HFPEF)
I.6 Stiffness and oxygen levels modulate common pathways in cultured mesenchymal stem cells - New tools for HIE treatment
I.7 Identification of large biomolecules as biomarkers of population lifestyle and industrial activities using environmental proteomics wastewater-based epidemiology (EP-WBE)
I.8 Proteome profiling of formalin-fixed paraffin-embedded (FFPE) tissue from stained histopathology glass slides
I.9 Label-free quantitative proteomics analysis reveals potential diagnostic biomarkers associated with osteoarthritis severity in human synovial fluid
I.10 Unveiling Parkinson's disease through discovery metabolomics

Poster Session II - Thursday, May 12th - 10.30

II.11 Serum proteomic profile of asthmatic patients after six months of benralizumab and mepolizumab treatment
II.12 Automated workflows for dia data using dia-nn on the paser platform
II.13 Label-free single cell analysis workflow on the timsTOF SCP mass spectrometer using the CellenONE platform
II.14 Absolute quantitative proteomics of chromophobe renal cell and renal oncocytoma
II.15 A new approach for easy and reliable Bladder Cancer Diagnostic and Monitoring
II.16 Autoimmune Hepatitis and Primary Biliary Cholangitis: bottom-up analysis of the acidic insoluble fraction of salivary proteome
II.17 Blood proteomic analysis of the effect of physical exercise in older adults
II.18 Exploring the role of secretome on somatic embryogenesis efficiency in Olea europaea L.
II.19 A mass spectrometry-based proteomics approach for FFPE laser-capture microdissected renal tissue analysis focused on Monoclonal Gammopathy of Clinical Significance
II.20 Exercise training modulates mitochondrial proteome plasticity in murine urothelial carcinoma-induced cardiac remodeling

Poster Session III - Thursday, May 12th - 15.50

III.21 Application of diaPASEF proteomics in the discovery of new lung cancer biomarkers in sputum
III.22 High-resolution MALDI-MSI workflow for proteomic analysis of zebrafish embryos
III.23 SUMO-ID: biotin-based identification of proximal SUMO-dependent interactors using mass spectrometry
III.24 Proteomics analysis of single human oocytes using magnetic beads based digestion and ultra-sensitive MS acquisition
III.25 Differential protein expression analysis of systemic lupus erythematosus to lupus nephritis evolution by label free lc-ms
III.26 Comparison of nitrate assimilation in three marine Synechococcus strains
III.27 Discovery of protein biomarkers for the diagnosis of equine metabolic syndrome
III.28 Identification of differential proteomic profiles in the synovial tissue of patients with rheumatoid arthritis and psoriatic arthritis
III.29 An affordable proteomic perspective on the search of sustainable plant-based proteins with technological applications in food industry
III.30 Protein interactions : how to decipher them?

Poster Session IV - Friday, May 13th - 9H50

IV.31 TMT-based quantitative proteomics analysis reveals differentially expressed proteins between different sources of hMSCs
IV.32 MS-based quantitative proteomic analysis of serum-purified exosomes for the identification of pre-eclampsia-associated biomarkers
IV.33 The serum of asymptomatic covid-19 individuals affects circulating angiogenic cells functions. a proteomic aproach.
IV.34 Proteomics Identification of Novel Amyloid-β Plaques Interactors Associated to Alzheimer's Disease
IV.35 Living donor liver transplant. understanding liver regeneration
IV.36 Seroprevalence of SARS-CoV-2 antibodies in healthcare workers and pediatric patients using a Luminex assay
IV.37 Upgrading X!TandemPipeline and MassChroQ for fast and accurate timsTOF native raw data support for quantitative proteomics
IV.38 The secretome of a fuel-ethanol Saccharomyces cerevisiae yeast starter during alcoholic fermentation – a preliminary study
IV.39 Deciphering the complexity of extracellular vesicles (EVs) mediated vascular calcification using a proteomic approach
IV.40 Rapid & scalable off-line peptide fractionation on zwitterionic magnetic microparticles
IV.41 Towards an innovative and effective production of recombinant proteases in tobacco BY-2 cells for the dairy industry

Symposium Abstracts

Pseudoscience and denialism in times of pandemic

Daniel Closa[1]

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There is a huge amount of information circulating on social media about products, activities or attitudes that are supposed to improve our lives. There are also those who warn us of possible threats or conspiracies that threaten our world. This includes flat earth advocates, anti-vaccine activists, sellers of supposedly miraculous therapies and deniers of anything we can imagine.

We usually didn't pay much attention to it and looked at it as a simple curiosity, sometimes irritating but anecdotal. But during the Covid19 pandemic it has become clear that some of these movements can have important consequences, especially in the field of health.

In a situation where even scientists may find it difficult to find out the reliability of news that appeared at an accelerated pace, it is not surprising that confusion has been generated among the general public. Unfortunately, this is a situation where sellers of conspiracies, pseudoscience, and different scams have a lot of ease in selling their scams.

There are different factors that explain the success of these movements. While science provides data and reasoning, pseudoscience and denialism sell emotions (fear, hope ...), which is an easier and effective strategy to attract followers. On the other hand, the historical reluctance of scientists to participate in these debates has allowed pseudoscience to take a center stage on social media, which does not reflect their minor importance in society. Nevertheless, the scientific community should not continue to ignore these movements and strategies should be put forward to counteract their impact on society's view of scientific knowledge.

Quantitative proteoform profiling of intact human protamines in infertile normozoospermic patients

Marina Gay[1], Judit Castillo[2], Gianluca Arauz-Garofalo[1], Alberto de la Iglesia[2], Mar Vilanova[1], Ada Soler-Ventura[2], Meritxell Jodar[2, 3], Marta Vilaseca[1], Rafael Oliva[2,3]

[1] Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Baldiri Reixac, 10, 08028 Barcelona, Spain. [2] Molecular Biology of Reproduction and Development Research Group, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Fundació Clínic per a la Recerca Biomèdica (FCRB), Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona (UB), 08036 Barcelona, Spain. [3] Biochemistry and Molecular Genetics Service, Hospital Clínic de Barcelona, 08036 Barcelona, Spain.

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Protamines play a crucial role in compacting the paternal genome by replacing histones as the main nuclear proteins in the sperm cells of many species. In humans, these proteins comprise protamine 1 (P1) and the family of protamine 2 (P2), which together pack the 85-95% of spermatozoa DNA. Alterations in protamine post-translational modifications (PTMs) or the P1/P2 ratio may be associated with male infertility. However, the specific deregulations that would result in an altered P1/P2 ratio have not yet been fully described.

Top-down proteomics enables high throughput analysis of intact proteoforms derived from missense or nonsense genetic variants, alternative splicing or PTMs. In contrast to current standard techniques, we established a top-down proteomics and search engines results integration using DBSCAN to obtain a more in-depth analysis of protamine proteoforms and their PTMs [1, 2].

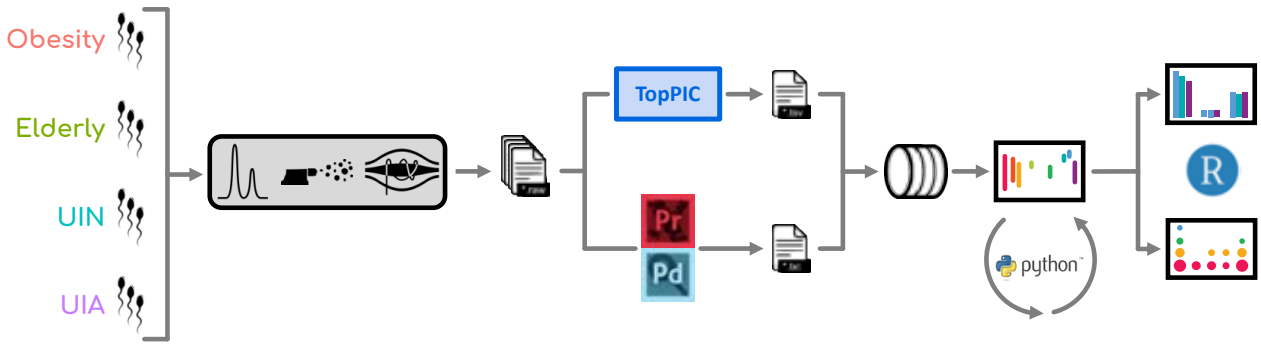
Here, we applied our top-down proteomics strategy to identify alterations in the protamine proteoforms profile in four types of infertile normozoospermic patients: elderly patients, obese patients and patients with Unknown Infertility with and without Altered P1/P2 ratio (UIA and UIN, respectively).

Taking UIN as reference patients, we found that two immature P2 proteoforms called HPS1 and HPI2 are more abundant in UIA patients, suggesting a disrupted processing of P2 in this phenotype. We also found that the particular phosphorylation profile of P1 might be linked with age-driven infertility.

Specific knowledge of these alterations will allow the development of strategies to identify and address new molecular causes of male infertility, opening up the potential personalized diagnosis and treatment of male infertility.

[1] Soler-Ventura, *et al. J Proteome Res. 2020 Jan 3;19(1):221-237.*

[2] Arauz-Garofalo, *et al.* Proteomes. 2021 Apr 30;9(2):21.



Proteoforms and their expanding role in biomedical research

Julia Chamot-Rooke[1], [1]Institut Pasteur, CNRS, Paris, France

Proteins are the primary effectors of function in biology, and thus, complete knowledge of their structure and properties is fundamental to deciphering function in basic and translational research. The chemical diversity of proteins is expressed in their many proteoforms, which result from combinations of genetic polymorphisms, RNA splice variants, and posttranslational modifications[1]. Addressing proteoforms is foundational to understand the biological complexes and networks that control biology. In this talk, the importance of proteoform-level knowledge in biomedical research is highlighted through several examples.

In multiple myeloma diseases, monoclonal immunoglobulin light chains (LCs) are abundantly produced, with, as a consequence in some cases, the formation of deposits affecting organs such as the kidney. The exact factors crucial for the solubility of LCs are poorly understood, but it can be hypothesized that their amino acid sequence plays an important role. Determining the precise sequences of patient-derived LCs is therefore highly desirable. To this aim, we established a novel *de novo* sequencing workflow based on the combination of bottom-up and top-down proteomics without database search. This pipeline was then used for the complete *de novo* sequencing of LCs extracted from the urine of 10 patients with multiple myeloma. We show that top-down proteomics is absolutely required to achieve 100% final sequence coverage and characterize clinical samples containing several LC proteoforms[2]. Our work highlights an unexpected range of modifications.

The current technique used for microbial identification in hospitals is MALDI-TOF MS. However, it suffers from important limitations, in particular for closely related species. We therefore set up a liquid chromatography (LC)-MS/MS top-down proteomics platform, which aims at discriminating closely related pathogenic bacteria through the identification of specific proteoforms[3, 4]. Using *E. coli* as a model, all steps of the workflow were optimized: protein extraction, on-line LC separation, MS method, and data analysis. We then used this platform for the discrimination of enterobacterial pathogens undistinguishable by MALDI-TOF, although leading to very different clinical outcomes. For each pathogen, we identified specific proteoforms that could potentially be used as biomarkers. We also improved the characterization of poorly described bacterial strains. Our results highlight the advantage of addressing proteoforms rather than peptides for accurate bacterial characterization and qualify top-down proteomics as a promising tool in clinical microbiology.

1. Smith, L. M.; Kelleher, N. L.; Consortium for Top Down, P., Proteoform: a single term describing protein complexity. *Nat Methods* **2013,** *10* (3), 186-7.
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Tackling Cancer Mechanisms with Spatially Resolved Proteomics

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Despite constant improvement in the patient care, solid tumors uptake is still difficult with high death rate and limited patient survival as well as resistance to treatment and recurrence. Thereof, better understanding of the pathophysiological mechanisms driving the initiation of cancer and its development is central to improve the treatment. One of the specific features of the tumors is their high heterogeneity both in terms of the different cell type involved, with the presence of the immune cells, and clonal heterogeneity of the cancer cells as well. This heterogeneity has surged the development of suited proteomic methodologies known as spatially resolved proteomics. Spatial proteomic information can be gathered by approaches ranging from MALDI MS Imaging to localized micro-extraction of proteins from tissue sections and the combination of them. These strategies were applied to the understanding of the development of ovarian cancer working from pre-cancerous ovarian lesions, clonal proteomics of breast cancer for treatment repurposing and to glioblastoma for patient stratification. Moreover, novel proteins issues from non-coding RNA and alternative open-reading frames in mRNA were unveiled from proteomic data. Very interestingly, these so-called Alternative Proteins (or ghost proteins) which represents a hidden part of the proteome are involved within the pathophysiological mechanisms. They indeed are shown to be involved among other in the regulation of different mechanisms. Various alternative proteins were identified in the different cancers thanks to the spatial proteomics and these represent interesting new diagnostic and prognostic markers as well as therapeutic targets.

Kinase signalling circuits in health and disease

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Cells need to constantly adapt to changes in conditions and use post-translational regulation as a fast way to transfer information from sensors to effectors of cellular responses. Advances in mass-spectrometry allow us to identify post-translational modification (PTMs) sites on a large scale and to quantify their changes across different conditions. However, the interpretation of these measured changes remains challenging. We have worked on approaches that try to predict the kinase-kinase regulatory network and how to use large scale phosphoproteomics to infer the activation state of kinases. As an example we have applied these approaches to study the changes in kinase signalling across tumour samples or occurring during SARS-CoV-2 infection. Based on the viral phosphorylation studies we could show how SARS-CoV-2 infection promoted casein kinase II (CK2) and p38 MAPK activation and the inhibition of cell-cycle kinases. These were linked to production of diverse cytokines, cell cycle arrest and stimulation of filopodial protrusions. Our work on the tumour samples revealed the kinases that are most often dysregulated in cancer and disconnect between the mutational status of the tumour and the predicted activation state of kinases, indicating substantial compensatory mechanisms. These approaches are starting to give us a less biased understanding of the kinase signalling network and uncovering the importance of phospho-regulation across multiple aspects of cell biology and disease.

How proteomics and advanced mass spectrometry are reshaping what we can learn about paintings, objects and cultural heritage

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For 20 years now, bio-mass spectrometry has been changing the analytical landscape of art, archaeology and cultural heritage. Alongside the technical improvements, the continuous emergence of new applications allows improved structural elucidation of ancient components and interacting biomolecules - as varied in structure as in their complexities, that are ancient lipids, polysaccharides and proteins. From a few micrograms of precious sample from an object, it is now possible to obtain protein identity sequences and to discriminate biological species on a single amino acid basis. In addition to this improved investigative process, enhancing knowledge of artworks / heritage objects and preservation approaches, we are now confronting, in heritage mass spectrometry, an even more complex issue of chemical decoding of biomolecular networks, their fine characterization, the study of cross-linking mechanisms, as well as the understanding of their modifications and interactions.

This presentation will illustrate how protein/lipid chemical signatures inform about ancient material manufacturing processes and conservation practices, and the impact of these procedures on macromolecules' structures. It will also illustrate how molecular information informs on societal, cultural and economic aspects of the past communities.

In this context, the presentation will describe our latest developments in bottom up, top-down and hydrogen deuterium exchange mass spectrometry to address complex questions of molecular interaction in networks in unaged and aged forms. Protein conformational changes and pigment interactions occurring during paint formulation, drying and ageing will be discussed. Analytical evidence of protein crosslinking in historic artworks and objects will be presented and discussed. Other examples will also demonstrate the whole mass spectrometry capabilities in elucidating restoration procedures, based on specific chemical signature monitoring. The presentation will show how our most recent miniaturized analytical procedures for trace level analysis have provided insight into artists' working methods or objects' provenance. Finally, the presentation will show how high resolution (lateral and mass) MALDI imaging can decode the biomolecular organization of paint layers by identifying and mapping both high and low molecular weight ions for molecular structure characterization. We will demonstrate the suitability of the technique to locate and monitor intact materials and by-products within samples, at the surface, within the layers and at layer interfaces of artworks.

These examples will be illustrated by several outstanding cases of study from the Metropolitan Museum of Art collection in the context of the ARCHE international laboratory - *ARt and Cultural HEritage: Natural Organic Polymers by Mass Spectrometry*. Painted textiles and storage jars from ancient Egypt, African sculptures and Power Figures, Egyptian ivories, Chimú feather work, Coptic manuscripts, and paintings spanning the 15th to the 20th centuries will be a part of this presentation.

Evasion of Cellular Anti-viral Defences Deciphered by TAILS N-terminomics Analysis of SARS CoV-2CLpro Substrates in COVID-19

Pablos, I., Machado, Y., de Jesus, H.C.R., Mohamud, Y., Kappelhoff, R., Lindskog, C., Vlok, M., Bell, P.A, Butler, G.S., Grin, P.M., Cao, Q.T., Nguyen, J.P., Solis, N., Abbina, S., Rut, W., Vederas, J.C., Szekely, L., Szakos, A., Drag, M., Kizhakkedathu, J., Mossman, K., Hirota, J., Jan, E., Lou, H., Banerjee, A., and **Overall, C.M.**

Transcriptomics identifies mRNA levels, but not protein levels—yet whereas proteomics identifies protein levels, these do not necessarily reflect biological activity of the protein. Protein N and C terminal peptides provide critical information on protein function and stability. Our terminomics methods (TAILS, PICS) enrich and annotate N- and C-terminomes, and our N- and C-termini database TopFIND v 4.1 (https://topfind.clip.msl.ubc.ca) reveal widespread truncation and generation of termini in normal and diseased tissues and associated change in protein functions. In the pandemic, we pivoted to understanding the pathogenesis of COVID-19 by identifying the SARS-CoV-2 3CLpro main protease human protein substrate repertoire. 3CLpro, the main viral protease, is indispensable for SARS-CoV-2 replication.

We delineate the interconnected human protein substrate landscape of 3CLpro using TAILS analysis of human lung and kidney cells treated or not with interferons (N = 12), supported by analyses of SARS-CoV-2-infected human lung cells. Over 100 substrate discoveries were identified and validated by MALDI-TOF analysis of synthetic peptide cleavage kinetics for each cleavage site in >100 substrates. Molecular docking simulations of 3CLpro engaging substrates confirmed the ~10% of validated sites were noncanonical that diverge from SARS-CoV (-1) to guide substrate and inhibitor drug specificity. PPI analysis shows that cleavage by 3CLpro of the interactors of essential effector proteins, effectively strands these from their binding partners, and so amplifies the consequences of proteolysis throughout the cell by protein complex disassembly. Using recombinant protein digestion, Edman degradation, and digestion of normal human bronchial epithelial cell from 5 subjects we further confirmed substrate cleavages. We show that 3CLpro targets multiple proteins in the Hippo pathway, including inactivation of MAP4K5, as well as key effectors of transcription, mRNA processing and translation. We discovered that SARS CoV-2 Spike protein directly binds galectin-8, cleavage of which disengages CALCOCO2/NDP52 and decouples protective anti-viral-autophagy. Indeed, unlike healthy lung, in post-mortem COVID-19 human lung samples obtained from the Tissue Human Protein Atlas, NDP-52 rarely colocalizes with galectin-8. Thus, great mechanistic insight to novel mechanisms of evasion of antiviral host cell immunity, hijacking of the host mRNA binding and translation apparatus, disruptions in cell shape and syncytium formation was enabled by rationale start points identified in the 3CLpro substrate degradome. The Atlas of CoV-2 substrates establishes a foundational resource to accelerate further exploration of SARS-CoV-2 pathology in the COVID-19 *cellular coup d'etat*.

Pablos, I., et al and Overall, C.M. 2021. Mechanistic Insights into COVID-19 by Global Analysis of the SARS-CoV-2 3CLpro Substrate Degradome. Cell Reports 37, 1–17
DOI:https://doi.org/10.1016/j.celrep.2021.109892.

Antigen Discovery for Development of Personalized Cancer Immunotherapy

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Abstract:

The remarkable clinical efficacy of the immune checkpoint blockade therapies has motivated researchers to discover immunogenic epitopes and exploit them for personalized vaccines and T cell based therapies. Mutated human leukocyte antigen binding peptides are currently the leading targets. Recently we have developed a dedicated computational pipeline called NeoDisc that can precisely characterize the antigenic landscape of tumors, incorporating whole exome sequencing, transcriptomics, mass spectrometry (MS) based immunopeptidomics and advanced HLA binding prediction tools. NeoDisc can lead to the direct (MS-based) identification of mutated neoantigens by MS as well as non-canonical cancer–specific peptides derived from unconventional coding sequences in the genome, and it predicts and prioritize the most likely immunogenic antigens. Since 2020, we apply this antigen discovery approach in cancer vaccine and adoptive transfer of neoantigen-specific T cells phase I clinical trials.

High-throughput phosphoproteomics to analyze cell signaling networks in health and disease

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Abstract:

Mass spectrometry-based is emerging as an indispensable tool for analyzing perturbed cellular signaling networks on a systems-wide scale. In this presentation, I will go through recent technology developments in proteomics sample preparation, LC-MS/MS acquisition strategies and data analysis, which enabled us to analyze cellular rewiring of spatiotemporal phosphoprotein signaling as a function of growth factor stimuli and drug treatment [1]. I will describe a new spatial phosphoproteomics technology that is based on a simple chemical fraction method for high-throughput and reproducible analysis of subcellular phosphoproteomes by using short LC gradients and data-independent acquisition (DIA). The subcellular analysis workflow is based on sequential cell fractionation to profile the global proteome and phosphoproteome dynamics across six distinct subcellular fractions. We applied the workflow to study spatio-temporal EGFR phospho-signaling dynamics in-vitro in HeLa cervix carcinoma cells and in-vivo in mouse tissues. We have also investigated the spatio-temporal stress signaling induced by osmotic shock in U20S osteosarcoma cells revealing cellular relocation of ribosomal proteins in response to hypertonicity and in muscle contraction. Most recent, we have used the workflow to study the impact of a cancer therapeutic drug on the subcellular (phospho)proteome in sensitive and resistant acute myeloid leukemia (AML) cells. Our spatial proteomics method is a powerful strategy for studying phospho-signaling dynamics at subcellular resolution.

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A travel guide to metaproteomics with tips for the study of the most complex samples

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Understanding the functioning of microbial communities requires identifying the diversity of microorganisms present and quantifying the numerous molecular players in action. Proteins are the workhorses of biological systems and metaproteomics is a key technology for such studies. The different facets of metaproteomics, including sample preparation, data acquisition, database construction, search strategy, and data interpretation, will be presented and commented. Several examples including soil, one of the most complex samples, will illustrate these facets.

Oral Presentations Abstracts

Parkinson's disease plasma biomarkers: from translational research to alternative proteomics approaches

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Background: Blood biomarker discovery has been dominated by targeted analysis of disease-associated proteins or conventional untargeted proteomics strategies. However, these attempts have failed to identify high confidence biomarkers of neurodegenerative diseases (NDs), which we believe is in part due to: i) the use of conventional proteomics analyses in a very challenging sample, such as blood, and ii) the difficulties in classifying the patients' groups.
Moreover, most of these studies are focused on a single fraction of the blood, such as plasma or serum, which only reveals an incomplete set of circulating proteins. Thus, the present work aimed at obtaining a comprehensive characterization of the circulatory proteins by combining different proteomics analyses of blood samples from the same individuals, including i) undepleted plasma; ii) peripheral blood mononuclear cells (PBMCs); iii) high and low molecular weight fractions of the plasma/serum, usually misrepresented in conventional analysis, and translational research starting with cell secretome and targeted data analysis from SWATH data of plasma samples.
Methodologies: To achieve this goal, state-of-the-art quantitative proteomics, SWATH-MS, was used in the four analyses referred above. These analyses were performed for a representative group of the two most common NDs and a control. The diagnostic model achieved was further tested in some potential Alzheimer's and Parkinson's disease cases.
Findings: From this combinatory study, it was possible to quantify thousands of proteins, with some capable of distinguishing the NDs from the control samples and AD from PD patients.
Conclusions: The results achieved in this preliminary study strengthened the importance of combining alternative proteomics strategies to obtain a deeper characterization of patient samples, creating a "blood signature", which has a higher potential as a source of biomarkers. Future work will comprise the introduction of more disease groups and test if protein complexes can be used to predict aging and age-related ND disorders.

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Proteomics and mass spectrometry imaging study of three-dimensional cell culture of breast tumor

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Breast cancer is the most frequent cancer among women, causing the greatest number of cancer-related deaths. Currently, breast cancer research is mostly carried out using 2D cultures or with mice models. 2D cultures have shown limitations, since they lack cell-cell and cell-matrix interactions and are generally composed of one cell type1. On the other side, in-vivo models (e.g. mice) are associated with ethical concerns, are intrinsically more complex, are expensive to establish and they do not accurately reflect human conditions. There is an unmet need to develop novel more accurate, realistic, standardisable and robust experimentation models. In addition, many studies of new drugs that have been shown to be highly effective on 2D cell lines, have failed in clinical trials. Is in this context that 3D cultures became interesting in research in order to mimic the in vivo conditions.

Is in this context, that we developed breast cancer organoids made from a mixture of tumor cells isolated from human and dog breast tumor tissues. We characterized these organoids by immunohistochemistry, HE staining. Then, for study in depth the characteristics of the tumoroids from tumors, we carry out the proteomics and mass spectrometry imaging study.

First we compare the tumor with the tumoroids that come from it. Our results reveal that organoids are very closed from the original tumor based on their protein profiles. Moreover, we realize mass spectrometry imaging in tumoroids and our results highlighted the intratumor heterogeneity of the tumoroids model which is very important to recapitulate in vivo responses to drugs. Thanks to these results, we became interested in the study of the different populations within the tumoroids that represent the intratumor heterogeneity. We recover different populations and we study by mass spectrometry imaging the similarities between the different populations and the tumoroid of origin and also we analyze the impact of the response to drugs between them.

Keywords: Organoids, bio-printing, breast cancer, proteomics, mass spectrometry imaging.

Sub optimal ^{15}N metabolic labelling in plant: A new way to perform large scale protein turnovers determination using the experimental isotopic distribution.

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Proteostasis is defined as the processes required maintaining the equilibrium between protein synthesis and degradation. Tight regulations from gene transcription to post-translational proteins modifications of these processes are required in cell cycle and survival to balance cellular development and environmental stresses. It has been shown repeatedly that the abundance of proteins only moderately correlates with that of transcripts. While the determination of protein turnover using pulsed SILAC metabolic labelling is popular for large scale proteomics investigations. Although used with small mammals (e.g. mice), such approach remains difficult with whole organisms and almost impossible with autotroph species like plants. Alternatively, it has been proposed to use inorganic sources of ^{15}N isotopes that could be metabolically incorporated to *de novo* synthetized amino acids but full ^{15}N protein labelling is almost impossible due to the high amino acid recycling rate in plant.

Instead of reaching complete $^{14}N/^{15}N$ exchange, we decide to perform a ^{15}N metabolic pulse at very low incorporation yield (5 - 10% ^{15}N final). This limited incorporation rate offers some advantages of which a limited increase of mass spectrometry signal complexity. Indeed, the ^{15}N-labelled products moderately modify the peptide natural isotopic distribution and both labelled and unlabelled product appears as an overlapping isotopic distribution. Then, the recorded signal is used to perform label free quantitation and to determine the “Protein Fold Change” (PFC) evolution over time. The experimental peptide isotopic distribution is used to determine the apparent ^{15}N labelling rate and the evolution of the ^{15}N “Labelled Protein Fraction” (LPF) over the labelling time.

Thanks to our local bioinformatics developments including i2MassChroQ, MassChroQ and the MCQR package, we propose an approach of MS signal processing that make it possible to determine the fold change in protein (FCP) and the labelled protein fraction (LPF) required to estimate protein turnover parameters {Li, 2017 #5309} i.e. protein half-life and synthesis/degradation rates at the proteomic scale. Furthermore, this presentation highlights that accurate isotopic distribution measurement is possible and could be used from ordinary large-scale proteomics LC-MS/MS instruments to provide an interesting alternative to determine protein turnover determination at the proteome scale.

Key words: protein turnover, bioinformatics, nitrogen stable isotope (15N), metabolic labelling, isotopic distribution, large scale proteomics.

Innovative multiplex proteotyping of microbial isolates

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Accurate and rapid identification of any microorganisms is essential to answer clinical diagnostic demand and microbiological screening in general starting from complex microbiota. To gain insights into microbiota individual components, culturomics has been proposed to test systematically hundreds of possible cultivation conditions and generate numerous microbial isolates with very different characteristics. Microbial diagnostics and culturomics rely until now on whole-cell MALDI-TOF-MS as gold standard method for microbial identification. The method is rapid and low-cost but presents some limits and drawbacks in the coverage of environmental species in the current experimental reference databases, the power for discriminating sub-species or even strains and clones, and cannot handle correctly the analysis of mixtures of microorganisms. Proteotyping by tandem mass spectrometry offers a much better level of identification of microorganisms by linking acquired MS/MS spectra associated to peptide sequences directly to species. We have developed a powerful approach, named "Phylopeptidomics"(1), which enables the taxonomic identification of microorganisms, even in complex mixtures and the quantitation of their relative biomasses. We have explored its applications on environmental and clinical isolates (2–4). Multiplexing samples prior to tandem mass spectrometry proteotyping could be an excellent mean to achieve high-throughput analysis and lower the associated costs.

Here, we explored an off-line fractionation of global peptidomes that could be applied on any microorganisms, allowing to combine different fractions from a large set of isolates and link the final result to the correct sample. The mixture of peptides corresponding to multiplexed microorganisms is then resolved by reverse phase chromatography and analyzed by tandem mass spectrometry in a single run. The complex dataset is then interpreted to identify the microorganisms present in the mixture and determine from which fraction they belong. As an example, 21 microorganisms could be identified in a single 60-min run performed with a Q-exactive HF high-resolution mass spectrometer, resulting in a rate of one microorganism identified per 3 min of mass spectrometry without labelling. Organisms of the same genus were easily distinguished as seen for *Pseudomonas*, *Bacillus* and *Deinococcoccus*. The results from this proof of concept open new perspectives for the application of high-throughput proteotyping of bacteria using tandem MS in culturomics large projects.

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Proteomic profiling of farmed gilthead seabream skin mucus stress response: a biomarker discovery study

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Stress management is crucial for farmed fish welfare as it is to ensure the sustainability of the aquaculture industry. The study of the fish stress response under routine procedures and certain intensive farming conditions, through the identification of unbiased species-specific molecular stress indicators is mandatory to better characterize a stressed state and to develop new and more reliable stress management protocols. Proteomics was employed in this study to investigate the gilthead seabream skin mucus proteome response to different challenges and as a tool to discover protein stress indicators. Fish skin mucus acts as the first line of defense of the innate immune system, and a few studies have already highlighted its potential for stress monitoring in different fish species, with the main advantage of allowing for sampling in a minimally invasive way.

Sparus aurata was exposed to different suboptimal rearing conditions in three separate trials: overcrowding, net handling, and hypoxia, using fish reared under optimal conditions for the species as control. Fish were sampled by the end of the trials, and mucus protein extracts were prepared for further analysis by reverse phase nano liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Proteins identified with high confidence (protein FDR <0.5%; peptide FDR <0.1%) within each trial were analyzed by One-way ANOVA followed by Tukey's HSD test ($p < 0.05$). Differential abundant proteins (DAP) were screened for protein-protein interactions and enriched KEGG and REACTOME pathways. Pairwise comparisons between control and stressed fish samples were also established within each trial (T-test, q <0.05), and up- and down-regulated proteins were further classified according to Gene Ontology Enrichment (Fisher's Exact test, FDR <0.05). The discriminative power of specific mucus proteins, selected by different feature selection methods, was assessed through ROC curves after logistic regression analysis.

Label-free shotgun proteomics reproducibly identified a mean of 1300 proteins in at least 4 out of 6 fish per treatment, in the skin mucus of gilthead seabream. A total of 250 DAP were identified between challenged and control fish across the three trials, mostly implicated in translation, protein folding processes and immune system. The ROC curve analysis showed that, among others, the mucus proteins haptoglobin and alpha-2-macroglobulin presented a high predictive power (AUC > 0.8) and could thus be further investigated as potential indicators of different stressors. Still, further verification and validation steps are required. This discovery approach provides a starting point for identifying reliable molecular lab-based welfare indicators for this species and to further improve rearing welfare protocols for higher responsible aquaculture.

Glycoproteogenomics Characterization of the CD44 Splicing Code in Bladder Cancer Towards Precision Oncology

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Bladder cancer (BC) management demands the introduction of novel molecular targets for precision medicine. Cell surface glycoprotein CD44 has been widely studied as a potential biomarker of BC aggressiveness and cancer stem cells. However, significant alternative splicing and multiple glycosylation generate a myriad of glycoproteoforms with potentially distinct functional roles. The lack of tools for precise molecular characterization has led to conflicting results, delaying clinical applications. Addressing these limitations, we have interrogated the transcriptome of a large BC patient cohort for splicing signatures. Remarkable CD44 heterogeneity was observed, as well as associations between short CD44 standard splicing isoform (CD44s), invasion and poor prognosis. In parallel, immunoassays showed that targeting short *O*-glycoforms linked to bladder cancer aggressiveness could hold the key to improve CD44 cancer specificity. This prompted the development of a novel glycoproteogenomics approach, building on the integration of transcriptomics-customized datasets and glycomics for protein annotation from nanoLC-ESI-CID/HCD-MS/MS experiments. The concept was applied to invasive human BC cell lines, glycoengineered cells presenting simple and homogeneous glycosylation, and tumor tissues, enabling unequivocal CD44s identification. Finally, we confirmed the link between CD44s and invasion *in vitro* by siRNA knockdown, supporting findings from BC tissues. The key role played by short-chain *O*-glycans in CD44-mediated invasion was also demonstrated through glycoengineered cell models. Overall, CD44s emerged as biomarker of poor prognosis and CD44 carrying truncated glycans as promising molecular signatures for targeted interventions. This study materializes the concept of glycoproteogenomics and provides a key vision to address the cancer splicing code at the protein level, which may now be expanded to better understand CD44 functional role in health and disease.

Acknowledgments

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Quantitative proteomics analysis of aryl-hydrocarbon receptor-interacting protein reveals a novel protein able to regulate tumorigenic and metastatic properties of colorectal cancer cells driving liver metastasis

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Isogenic cancer cell models with different metastatic capacities in combination proteomic analysis have proven to be a tremendously powerful tool for the identification of diagnostic markers and new targets of intervention. These types of studies provide with heaps of information about proteins upregulated in cell lines with metastatic potential. In this work, we have aimed to shed some light on one of the proteins – Aryl-hydrocarbon receptor interacting proteins (AIP)- found to be upregulated in the KM12SM metastatic to liver colorectal cancer (CRC) cells compared to the isogenic KM12C non-metastatic CRC cells.
In silico analysis of AIP expression revealed a significant association between high AIP mRNA levels and worse CRC patient survival. In parallel, TMA analysis of colorectal cancer samples from patients also showed correlation between the probability of relapse and high AIP expression levels. To understand in detail the role of AIP in the metastatic progression of CRC, we ectopically overexpressed AIP in both KM12C and KM12SM CRC cells.
Ectopic expression of AIP in non-metastatic KM12C cells lead to a drastic change in the metastatic capacities of these cells. We observed significant increases in wound healing, adhesion, invasion and colony formation capacity of KM12C cells when AIP was overexpressed.
Quantitative TMT 10-plex proteomic analysis of protein extracts from cells over expressing AIP showed 37 upregulated and 23 commonly downregulated proteins in KM12C and KM12SM upon AIP ectopic expression, as EGFR and CDH17 which induce a metastatic phenotype in the cells.
AIP overexpression also induced changes in protein and mRNA levels of EMT mediators such as E-Cadherin o TGF-ß, driving changes in localization of E-Cadherin and ZO-1 in both KM12C and KM12SM cells. The higher metastatic phenotype induced upon AIP ectopic expression was corroborated in vivo. KM12C cells over expressing AIP were able to colonize the liver of nude mice while the mock counterpart did not show any colonization potential. Additionally, there was a significant drop in the survival rate of the mice injected with KM12C over expressing AIP compared with the mock controls.
Taken altogether, proteomics analyses followed by in vitro and in vivo analysis revealed new roles for AIP in regulating proteins associated with cancer and metastasis, which collectively induced higher tumorigenic and metastatic properties in colon cancer cells driving liver metastasis.

Keywords: colorectal cancer; metastasis; quantitative proteomics; TMT; mass-spectrometry.

Proteomic profiling of ascending aortic VSMC reveals alterations in DNA machinery and cell cycle dysregulation in thoracic aortic aneurysm associated with bicuspid aortic valve

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Background and aim: Thoracic aortic aneurysm (TAA) is a silent and life-threatening disease caused by the progressive dilatation of the aortic wall. The vast majority of TAA are non-syndromic non-familial TAA, but its incidence is significantly higher in bicuspid aortic valve (BAV) subjects for unknown reasons. Diagnosis is fortuitous and by expensive imaging tests, and the only effective treatment is surgery, partly due to the limited knowledge of the pathogenic mechanisms. Our aim is two-fold: 1) deciphering protein changes subjacent to TAA associated with BAV; and 2) identifying a protein profile reflecting TAA formation with diagnostic potential. Considering that vascular smooth muscle cells (VSMCs) are essential for the maintenance of the aortic wall structural and mechanical integrity, we analyzed the proteome of these cells. Main changes taking place within the aortic structure may ultimately be translated into plasma so we additionally evaluated potential biomarkers in plasma samples.

Methods: Patients with programmed surgery due to thoracic aorta dilatation or valvulopathy or both were recruited and classified in TAA (with BAV or tricuspid aortic valve (TAV)) or control. Human VSMCs were isolated from 5 BAV-TAA and 4 TAV-TAA aortas. A total of $8x10^6$ cells were analyzed per sample by TMT isobaric labeling and LC-MS/MS. Systems biology analysis was performed to reveal key biological processes happening. Differential proteins potentially secreted extracellularly were further investigated by targeted analysis in 82 plasma samples from TAA and control subjects.

Results: 21 stress-related proteins were significantly altered between BAV and TAV VSMCs pointing to them being exposed to different biomechanical stresses. Alterations in the characteristic constituents of the extracellular matrix (ECM) revealed a decrease in ECM interactions together with decreased cellular adhesion. Coordinated protein behavior showed that processes related with DNA and cell cycle were decreased in BAV patients, reflecting an impairment in DNA replication and in the response of the machinery to DNA damage, alterations in DNA structure and defective protein-strain binding. On the contrary, processes involved in vascular remodeling, cell transduction and motility, G protein and Ephrin B signaling and cleavage of phospholipids were upregulated. Translational plasma analyses revealed the existence of three diagnostic markers which also correlate with the aortic diameter.

Conclusions: Changes identified in human VSMCs point to a defective repair capacity of the aortic wall in subjects with BAV. The discovery of three novel plasma TAA biomarkers could aid in the early diagnosis/prognosis of TAA.

Looking for new plasma biomarkers of Non-Alcoholic Fatty Liver Disease (NAFLD) progression using discovery and targeted proteomics

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Liver diseases are a major global health problem, causing more than 2 million deaths per year worldwide[1]. Most liver diseases result from viral infections, alcoholism and NAFLD (Non-Alcoholic Fatty Liver Disease). NAFLD is highly prevalent (25% of the global population worldwide [1, 2]) and closely associated to the rise of obesity in the world[1]. Patients with NAFLD may progressively develop NASH (Non-Alcoholic Steato-Hepatitis) requiring liver transplantation in the most advanced stages[3,4]. Liver biopsy remains the reference examination to detect NAFLD, but it is an expensive, invasive and inappropriate tool for massive screening[3]. Although a large number of potential biomarkers has been discovered for NAFLD patient stratification, none of them is presently able to reach the performance of liver biopsy and histopathological examination. The development of non-invasive tests to diagnose NAFLD patients from the earliest stage is still a crucial need.

As a contribution to the field, we carried out a LC-MS/MS proteomic discovery study using plasma samples (n=160) from NAFLD patients diagnosed at Grenoble hospital hepatology department and classified into five groups of disease severity (fibrosis progression), according to liver biopsy and histopathological examination. This discovery study led to the identification of 114 plasma proteins with differential abundances between the five groups of NAFLD patients.

Among these 114 putative biomarkers for patient stratification, we selected 15 proteins for further evaluation based on two criteria: (1) a specific expression in the liver and (2) an absence of previous description as NAFLD biomarker in the literature. Then, these 15 proteins were precisely quantified in the 160 plasma samples obtained from NAFLD patients. For this purpose, a list of 33 signature peptides was established and a scheduled SRM assay was optimized using AQUA peptides as quantification standards. These quantitative analyses confirmed two plasma proteins as potential biomarkers to discriminate the severity stages of NAFLD.

In order to verify the relevance of our results, a validation study targeting these two plasma proteins will be launched by SRM and ELISA (enzyme-linked immunosorbent assay) in an independent cohort of NAFLD patients (n=200).

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Identification Of Novel Dysregulated Proteins And Amyloid-β Plaques Interactors Associated To Alzheimer's Disease By Proteomics.

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Introduction and objective

Alzheimer's disease (AD) is a progressive, chronic and neurodegenerative disease, and the most common form of dementia worldwide. Still, the mechanisms underlying the disease are not well known, and thus, the study of proteins involved in its pathogenesis would allow getting further insights into the disease and identifying new potential markers of AD. We here aimed to analyze protein dysregulation in AD tissue by quantitative proteomics to identify proteins that could play a major role in the disease.

Methods

TMT (Tandem Mass Tags) 10-Plex-based quantitative proteomics experiments were performed using frozen tissue samples from the left prefrontal cortex of AD patients at Braak stages IV-VI, and healthy individuals and patients with other dementias (vascular and frontotemporal dementia) as controls. LC-MS/MS analyses were performed on a Q-Exactive, and data analysis was performed using MaxQuant and Perseus to identify differentially expressed proteins in AD.

Result and discussion

In total, 3281 proteins were identified and quantified in the prefrontal cortex. After data analysis with Perseus, we observed 31 and 250 proteins upregulated and downregulated, respectively, in AD patients in comparison to controls, with a fold change ≥ 1.5 or ≤ 0.67 at two or more Braak stages. After bioinformatics analysis, we selected 10 proteins dysregulated in AD to study their role in the disease. Their dysregulation in the disease was verified by orthogonal techniques (qPCR, WB, IHC, IF and ELISA) using tissue and serum samples of AD patients, and patients with other dementias and healthy individuals as controls.

Conclusion

TMT-based quantitative proteomic experiments allowed the identification of proteins altered in AD previously and non-previously related to the disease. The dysregulation of these proteins at mRNA and protein level was confirmed in AD patients, highlighting a major role of these proteins in the development of the disease. One protein showed diagnostic ability of the disease as blood-based biomarker, and two proteins were identified as novel Amyloid-β plaques interactors.

Keywords: Alzheimer's disease, Proteomics, 10-Plex TMT, Dysregulated proteins, Biomarkers, Amyloid-β plaques, Blood-based biomarkers.

Differences and similarities on metabolic response to pH by commensal and pathogenic *Staphylococcus epidermidis* strains

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Staphylococcus epidermidis is the main human skin commensal, however, when the host is immunocompromized and there is a skin barrier break, this bacterium can convert into a life-threatening pathogen associated to medical devices-related infections. *S. epidermidis* population structure is composed of two genetic lineages (clonal complex 2 (CC2) and non-CC2) that co-exist in the human skin. CC2 strains are more frequently found causing disease, but the basis of this increased pathogenicity is still poorly understood. Improved knowledge of *S. epidermidis* pathogenicity mechanisms is needed to be able to design more effective prevention and treatment strategies.
To address this issue, we compared the adjustments of the metabolic and proteomic processes performed by CC2 and non-CC2 strains when facing pH stimulus that mimic skin (pH=5.5) and blood (pH=7.4) - a key contrasting environmental factor during commensal-to-pathogen conversion. Proteomics and metabolomics of cellular extracts recurred to nanoscale liquid chromatography- tandem mass spectrometry (nano LC-MS/MS) and ^{1}H NMR, respectively.
We observed that both strains shared similar metabolic adaptations to the low skin pH, such as an increase in glycerolipid metabolism and a decrease in metabolites involved in the two-component regulatory system SaeRS. On the other hand, they showed distinctive and specific responses to the two pHs tested. **At skin pH** CC2 strain promoted menaquinone biosynthesis and peptidoglycan-related pathways while non-CC2 strain, increased polyphosphate metabolism, betaine and folate biosynthesis. **At blood pH** CC2 strain showed a higher abundance of proteins that manage heme toxicity and adhesion while non-CC2 strain altered his carbohydrate-active transport system, butonate, arginine and proline metabolism.
Our results showed that CC2 and non-CC2 strains resort to distinct metabolic and cellular processes to cope with skin and blood pH. Contact of CC2 strain with blood pH induced metabolic pathways that allow survival in blood and can promote adhesion to medical devices. Data gathered suggest that acidification of the infection site and inhibition of heme detoxification and adhesion might be effective treatment strategies against *S. epidermidis* infections.

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SARS-CoV-2 natural immunity to vaccine-induced immunity: Systematic Evaluation of Humoral Response by multipronged functional proteomics approaches

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- **Abstract text**:

An unresolved question is to find out whether the hybrid immune model (natural infection plus vaccine administration or vaccine plus infection) can be a reproducible method to boost immunity. In both cases, dynamic integration of multiple components of the humoral immune response is required, among the cellular immunity. Here, we present a comprehensive and systematic analysis of humoral response against SARS-CoV-2 antigens in recovering COVID19 patients and vaccine induced immune response based on multipronged functional proteomic approaches. Immunoglobulin profile was assed against SARS-CoV-2 virus in a full proteome epitope array including the main structural proteins, accessory and non-structural proteins by full-length structure and epitopes peptides. Additionally, the acute phase reactants and antibody profiles were characterized by protein microarrays in order to further investigate the humoral response generated by the SARS-CoV-2 infection. The elucidation of common hallmarks of immunogenic epitope production in vaccinated and naturally protected individuals can bring valuable insights to reveal the immune processes underlying favorable disease progression. Our results can predict the increase in the intensity and breadth of the antibody response after vaccination in people previously infected with SARS-CoV-2 and previously vaccinated and follow infected by SARS-CoV-2

Exploiting Glycoengineered Simple Cells and Bioinformatics-Assisted Glycoproteomics for Bladder Cancer Targets Prioritization

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Bladder cancer (BC) faces limited therapeutic options, with more than 570,000 new cases and 200,000 deaths being reported in 2020, worldwide [1]. The sugar coating at the surface of cancer cells constitutes a promising source of cancer neoantigens, towards precision oncology. Aberrant glycosylation is a prominent feature of cancer, but with limited cancer specificity, due to its expression in non-malignant/pathological conditions [2, 3]. Therefore, interrogating the cancer glycoproteome may provide the bi-specificity necessary for targeted therapeutics [4].

Nevertheless, glycosylation heterogeneity still poses a challenge for proteome identification by mass spectrometry [5, 6]. Addressing this issue, we have started by applying a glycoengineering strategy based on CRISPR-Cas9 technology to our aggressive BC cell model, reducing the complexity of *O*-glycosylation to the simplest and cancer-associated *O*-glycan, Tn. Glycoproteomic analysis based on enrichment for plasma membrane proteins expressing this glycan resulted in the identification of more than 5,700 proteins, using the SequestHT search engine. This list of proteins was then used to generate a customized database for subsequent *O*-glycoprotein validation following lectin affinity chromatography for Tn-glycopeptides enrichment. Due to the difficulties with *O*-glycopeptide identification presented by conventional proteomics search engines, we have opted for the O-pair search algorithm, inbuilt in MetaMorpheus [7], which was used for confirmation of *O*-glycosylated proteins. Over 170 glycoproteins carrying the Tn antigen were identified with confidence and ranked according to their potential for clinical applications, namely targeted therapeutics, using an in-house developed algorithm [8]. Validation in patient samples is now required, envisaging clinical translation.

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The ghost proteome an hidden vision of the protein landscape

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It has been conventional accepted that mature messenger eukaryotes of ribonucleic acids (mRNA) are monocistronic leading to the translation of a single protein. However, large-scale proteomics have led to a massive identification of proteins from alternative open reading frames (AltORFs) translated from mRNAs at the 3'&5' UTR or by a shift of the CDS (+1 or +2) in addition to the predicted proteins issued from the reference open reading frame (RefORF) but also recovered from non-coding RNAs (ncRNAs). These alternative proteins (AltProts) are not represented in the classic protein databases and this "Ghost proteome" has not been considered until recently. However, these proteins were shown to be functional and there are growing evidences that they are involved in central functions such as cellular regulation both in the physiological and physiopathological context. Ghost proteins, therefore, represent a novel world, filling the gap in the understanding of signalling pathways, establishing as new markers of pathologies and therapeutic targets.

To open the vision of the proteomic landscape, I proposed a large description of our studies, mainly focused on biomarker research in cancer. I propose to address the findings made in clinical and research studies on the presence of several AltProt in breast cancer, and in particular certain observation of membrane AltProt maybe the next generation target for CAR therapy. In the pathology of Glioma, a large number of ncRNAs present in their sequences an ORF allow their translations into AltProt. It has been demonstrated that AltProts are involved in protein-protein interaction (PPI) with reference proteins (RefProt) reflecting their participation in signaling pathways related to cell mobility and regulation of transfer RNA. Most recently, clinical studies have found that AltProt also plays a role in the patient's survival and poor prognosis. A presentation of our observation in ovarian cancer will also be approached on the basis of the differential study of 3 cancer cell lines also involving a study of PPIs. Finally, I propose to present the presence of AltProt involved in the pathology of COVID-19, partners of viral proteins as well as a potential unreferenced expression of viral RNA.

The proteome of urinary extracellular vesicles reveals early sub-clinical cardiorenal risk in hypertensive subjects

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Aim: High albuminuria (urinary albumin to creatine ratio, ACR>30mg/g) is an indicator of cardiovascular and renal risk. Despite clinical evidences of early cardiorenal risk in normoalbuminuric subjects (ACR<30mg/g), they are out of clinical surveillance. Complementary to our previous study in urine [1], we aimed to identify alterations in urinary extracellular vesicles (uEVs) which may aid in assessing individual risk while revealing pathophysiological processes behind early albuminuria development.

Methods: Hypertensive patients were classified based on their ACR values as control (C) (ACR<10 mg/g) and High-normal (HN) (ACR=10-30 mg/g) (n=10). uEVs were isolated by ultracentrifugation and characterized by Western blot, nanoparticle tracking analysis and electron microscopy. uEVs proteins involved in albuminuria development were analyzed by isobaric-labeling (TMT) and LC-MS/MS. Systems biology analysis was carried out and coordinated protein responses were evaluated. Identified proteins and functional categories were considered significant if p-value<0.05. Targeted protein analysis in human renal tissue was additionally performed by immunohistochemistry to evaluate in situ renal tissue alterations and the potential in diagnosis of uEVs. A comparative analysis of the protein variations here identified in uEVs with previous data from our laboratory in urine from the same cohort was performed [1].

Results: A total of 480 proteins and 263 categories were found significantly varied in HN subjects vs C. A subset of 49 proteins, previously detected in urine but without significance, showed significantly altered abundance in uEVs from HN subjects reflecting altered homeostasis, coagulation cascade and lipids metabolism deregulation. Proteins of interest were also identified in tubular and glomerular components of human kidney.

Conclusion: uEVs constitute a valuable source in diagnosis of renal diseases, implementing the information available from urine. uEVs proteins evidenced early cardiorenal damage within the normoalbuminuria condition paving the way towards a personalized medicine in the control of cardiovascular risk.

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Does diabetes mellitus change the protein pattern in patients with aortic stenosis?

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Aortic stenosis (AS) and diabetes mellitus (DM) are both progressive diseases that if left untreated, result in significant morbidity and mortality. Several studies revealed that the prevalence of DM is substantially higher in patients with AS and, thus, the progression from mild to severe AS is greater in those patients with DM. Our goal is to study the molecular mechanisms implicated in the pathogenesis underlying both diseases and the relationships between them.

In this work, we analyzed aortic valve tissue, calcified and non-calcified, from patients with AS and with or without DM (n=4/group) by Tandem Mass Tag (TMT). In a first analysis, we compared the fold change calcified/non-calcified from the same patient between DM vs non-DM to study how diabetes affects calcification. Then, differentially expressed proteins were analyzed in plasma samples from an independent cohort of patients by Parallel Reaction Monitoring (PRM) (n=8/group), Enzyme-Linked Immunosorbent Assay (ELISA) or Western blotting (n=17/group) to corroborate their potential as diagnostic.

A total of ten differentially expressed proteins were confirmed in plasma by three orthogonal methods (PRM, ELISA or Western blotting). It is important to highlight that proteins confirmed by PRM were also confirmed by ELISA in order to check the power of mass spectrometry in translational research. Differentially expressed proteins are implicated in biological processes such as endopeptidase activity, lipid metabolism, coagulation and fibrinolysis or complement regulation. ROC curves were performed for these proteins in order to establish its sensibility and specificity. The diagnostic power of all proteins as a panel was much better than the proteins alone.

The results obtained in the present study provide the evidence that DM affects valve calcification, modifying the protein profile of aortic valve tissue. This finding may help to increase our understanding of the pathogenesis and on how DM affects the evolution of AS at molecular level.

THE HISTONE METHYL TRANSFERASE DOT1L REGULATES CHROMATIN REORGANIZATION AND MITOCHONDRIAL ACTIVITY, AND INTERACTS WITH THE PYRUVATE DEHYDROGENASE COMPLEX IN DIFFERENTIATING MALE GERM CELLS

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In mammals, sperm differentiation involves dramatic morphological and functional changes of germ cells, associated with a highly dynamic genetic program and extensive remodeling of their chromatin. Towards the end of spermatogenesis, male germ cell chromatin is profoundly remodeled, resulting in the eviction of ~85-99% nuclear histones and their replacement by more basic, smaller proteins, called protamines. This unique chromatin reorganization induces a 6-10 times higher level of compaction of the sperm chromatin compared to the canonical nucleosome-based chromatin.

We investigated by a multi-omics approach the role of the methyltransferase DOT1L and the consequences of its absence in male germ cells. DOT1L-KO impairs spermatogenesis and leads to defects in sperm motility and flagellum morphology. By RNA-seq analysis, we observed the deregulation of genes involved in flagellum organization and development as early as in primary spermatocytes. We also found that genes related to ATP synthesis, electron transport and oxidative phosphorylation are deregulated. We observed that DOT1L-KO in male germ cells impairs spermatozoa chromatin organization, as their chromatin is less compact, characterized by a higher proportion of retained histones, which reflects a deficient chromatin remodelling process in spermatids. In WT spermatids, prior to histone removal, histones – in particular histone H4 – are hyper-acetylated, and this phenomenon has been described by many studies to be a prerequisite for histone-to-protamine transition to proceed normally, as it creates a permissive state for histone removal. By proteomic analysis of histones, we observed that in DOT1L-KO spermatids, both H3K79 methylation and H4 hyper-acetylation were drastically reduced. By proteomic analysis of the proteins binding *in vivo* onto DOT1L, we identified the Pyruvate Dehydrogenase Complex, the mitochondrial complex responsible for the biosynthesis of acetyl-CoA from pyruvate. Further, by metabolomic analyses, we also found that DOT1L-KO in male germ cells affects acyl-CoA levels, including an increase in global acetyl-CoA and other acyl-CoA levels in DOT1L-KO compared to WT germ cells. The observation that DOT1L-KO disturbs acyl-CoA concentrations may have relevance beyond mitochondria activity since acyl-CoA are precursors of histone (and other proteins) post-translational modifications. In that respect, it is intriguing to observe that, in round spermatids, several histone H3 or H4 lysines have an increase in acetylation or crotonylation (for instance H3K18cr, H3K27ac, H4K8cr, K12ac, K16ac or H4K79ac). In summary, we have combined genomic, proteomic and metabolomic analyses to decipher the impact of the methyltransferase DOT1L on male germ cell differentiation into sperm.

Flash Oral Presentations Abstracts

ABSOLUTE QUANTITATIVE PROTEOMICS USING THE TOTAL PROTEIN APPROACH TO IDENTIFY NOVEL CLINICAL IMMUNOHISTOCHEMICAL MARKERS IN RENAL NEOPLASMS

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Abstract

Renal neoplasms encompass a variety of malignant and benign tumors, including many with shared characteristics. The diagnosis of these renal neoplasms remains challenging with currently available tools. In this work, we demonstrate the total protein approach (TPA) based on high-resolution mass spectrometry (MS) as a tool to improve the accuracy of renal neoplasm diagnosis. Frozen tissue biopsies of human renal tissues [clear cell (n = 7), papillary (n = 5), chromophobe (n = 5), and renal oncocytoma (n = 5)] were collected for proteome analysis. Normal adjacent renal tissue (NAT, n = 5) was used as a control. Proteins were extracted and digested using trypsin, and the digested proteomes were analyzed by label-free mass spectrometry. Quantitative analysis was performed by comparison between protein abundances of tumors and NAT specimens, and the label-free and standard-free TPA was used to obtain absolute protein concentrations. A total of 205 differentially expressed proteins with the potential to distinguish the renal neoplasms were found. Of these proteins, a TPA-based panel of 24, including known and new biomarkers, was selected as the best candidates to differentiate the neoplasms. As proof of concept, the diagnostic potential of PLIN2, TUBB3, LAMP1, and HK1 was validated using semi-quantitative immunohistochemistry with a total of 128 samples assessed on tissue micro-arrays. We demonstrate the utility of combining high-resolution MS and the TPA as potential new diagnostic tool in the pathology of renal neoplasms. A similar TPA approach may be implemented in any cancer study with solid biopsies [1].

Acknowledgments

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High-throughput and high-sensitivity biomarker monitoring in body fluid by FAIMS-enhanced fast LC SureQuant™ IS targeted quantitation

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Targeted proteomics methods have been greatly improved and refined over the last decade and are becoming increasingly the method of choice in protein and peptide quantitative assays. Despite the tremendous progress, targeted proteomics assays still suffer from inadequate sensitivity for lower abundant proteins and throughput, especially in complex biological samples. These attributes are essential for establishing targeted proteomics methods at the forefront of clinical use. Here, we report an assay utilizing the SureQuant™ internal standard triggered targeted method on a newest generation mass spectrometer coupled with a FAIMS (high-field asymmetric waveform ion mobility spectrometry) interface ion mobility device and an EvoSep One liquid chromatography platform, which displays markedly enhanced sensitivity and a high throughput of 100 samples per day. We demonstrate the robustness of this method by quantifying proteins ranging six orders of magnitude in human wound fluid exudates, a biological fluid that exhibits sample complexity and composition similar to plasma. Among the targets quantified were low-abundance proteins such at TNF-α and IL-1β, highlighting the value of this method in the quantification of trace amounts of invaluable biomarkers that were until recently hardly accessible by targeted proteomics approaches.

We applied this new workflow to gain better insight into molecular mechanisms of venous leg ulcers (VLU) that represent a clinical challenge and impair the patient's quality of life. We report the longitudinal protein expression pattern for nine wound biomarkers in VLU wound fluids from 57 patients treated with a protease-modulating polyacrylate wound dressing for 12 weeks and compare these with exudates from 10 acute split-thickness wounds. Measuring a total of 365 samples in less than four days, this analysis helped to assess healing responses upon treatment with a polyacrylate dressing.

Taken together, this method extends the toolkit of targeted proteomics assays and will help to drive forward mass spectrometry-based proteomics biomarker quantification.

1: Kalogeropoulos K, Savickas S, Haack AM, Larsen CA, Mikosiński J, Schoof E, Smola H, Bundgaard L, auf dem Keller U. High-throughput and high-sensitivity biomarker monitoring in body fluid by FAIMS-enhanced fast LC SureQuant™ IS targeted quantitation. bioRxiv 2022.03.22.485266; doi: 10.1101/2022.03.22.485266

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COMPARED DIA-PASEF AND PRM-PASEF APPROACHES FOR THE ABSOLUTE QUANTIFICATION OF 500 HUMAN PLASMA PROTEINS IN COLON CANCER PLASMA SAMPLES.

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Introduction

Targeted proteomics methods are a traditional choice for absolute protein quantitation of proteins in biofluids, as they allow to obtain a high selectivity, specificity and sensitivity while resolving the missing value problem for a limited number of targets. We recently evaluated the prm-PASEF acquisition strategy which allows to further increase the number of addressable targets and the method's selectivity without compromising the sensitivity. In parallel, if the dia-PASEF approaches now widely used to address the missing value problem in discovery studies have theoretically a reduced specificity, they have however a greater multiplexing potential, we are now applying both approaches to the absolute quantitation of 500 blood proteins in colon cancer plasma samples.

Methods

The plasma sample cohort consisted in 10 patients affected by a colon cancer (adeno carcinoma) and 10 controls. Plasma samples were depleted with a Mars 14 depletion column (Agilent), digested with a trypsin protease and spiked with a mixture of 800 quantified synthetic peptides (PQ500, Biognosys). All samples and controls were separated by nano-HPLC (nanoElute, Bruker Daltonics) on a 25cm X 75µm pulled emitter column (IonOpticks, Australia) packed with 1,6µm particles using a 100 min gradient. Peptides were analyzed on a timsTOF Pro instrument (Bruker Daltonics) operated both in prm-PASEF and dia-PASEF modes. Data processing has been done with Spectronaut (Biognosys), MaxQuant and Skyline-daily.

Results

We evaluated the quantification performance of the prm-PASEF in depleted plasma samples by monitoring 1564 precursor-ions corresponding to 782 peptides from 565 proteins while using a 2 min retention time window. The median relative standard deviation of the signal of the peptides was of 3%. We demonstrated accuracy over more than 3 orders of magnitude of peptides concentrations with a maximum error on the determination of 20%. 98% of the 574 quantified peptide pairs could be quantified from the prm-PASEF experiment, while 96% could be quantified from the dia-PASEF experiment. The results obtained by both approaches were highly correlated. The limit of quantitation obtained from the prm-PASEF experiment was down to 7.4 amol on column whereas it was down to 20 amol on column with dia-PASEF.

Conclusions

Both approaches have been successfully applied to the analysis of colon cancer plasma samples and allowed to spot regulated proteins that had formerly been spotted from cancer tissue analysis.

Metaproteomics analysis of gut microbiota: different processing methods allow the detection of specific phyla

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Metaproteomics is the study of all the proteins present in a biological sample at a given time point. The use of this technique for the study of the gut microbiota not only allows to decipher the taxonomic profile of the microbial communities present there, but also to have a global view of the different metabolic functions developed by the microbiota at that time. In this work, different methods for the initial sample processing and the disruption of microorganisms, which are both critical steps, were evaluated. The combination of a longer disintegration period of the stool sample, together with a sonication step increases the total number of peptides and proteins identified. Proteobacteria, Bacteroidetes, Planctomycetes, and Euryarchaeota identification was favored by mechanical cell disruption with glass beads. In contrast, the relative abundance of Firmicutes, Actinobacteria, and Fusobacteria was improved when sonication was performed before bead beating. Also, it was also observed that the use of sonication produces a detriment in the identification of human proteins.

To test the method, we analysed six samples from healthy individuals choosing a protocol that had render good results regarding taxonomic diversity and identification of proteins from Proteobacteria and humans. In relation to the functional analysis of microbial proteins relevant to the host, we identified proteins involved in vitamin B12 transport and metabolism, and short chain fatty acid (SCFA) production carried out mainly by members in the *Prevotella* genus and the Firmicutes phylum, respectively. Finally, we also detected 92 human proteins, which were mostly of them related to the antimicrobial humoral response.

The taxonomic and functional profiles obtained with the different protocols described in this work provide researchers with the necessary information to choose the appropriate protocol for the study of certain diseases that are suspected to be related to specific microorganisms of the gastrointestinal microbiota.

(García-Durán C, et al., 2021. Distinct Human Gut Microbial Taxonomic Signatures Uncovered With Different Sample Processing and Microbial Cell Disruption Methods for Metaproteomic Analysis. Front. Microbiol. 12:618566. doi: 10.3389/fmicb.2021.618566)

Undiscovery of regulators of senescence-associated secretory phenotype mediated by small extracellular vesicles

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Introduction: Cellular senescence is a process characterized by a stable cell-cycle arrest and the capacity to modify the microenvironment through the senescence-associated secretory phenotype (SASP), where cytokines, extracellular matrix proteins and proteases, as well as other factors that alter the behaviour of neighbouring cell have been found driving aging and age-related diseases. The enrichment in the number of senescent cells was observed *in vivo* during natural process like aging and development as well as pathological ones like cancer, fibrosis and wound healing. Small extracellular vesicles (sEV) are particles released by cells whose content is a mirror of the cells they come from. sEV are a new fashion intercellular communication way. In the last year, we reported that sEV are involved in the paracrine senescence transmission, denominated non-classical SASP. They have high potential to develop senomorphics, which are drugs that modulate SASP to treat age-related diseases as cardiovascular diseases, Type 2 diabetes, pulmonary fibrosis...
Aim: In this project, we want to find a proteomic signature of the emerging non-classical SASP mediated by sEV. We are focusing to undiscover pathways involved in the SASP through sEV.
Methodology: For that, we performed the identification, quantification, and comparison of proteome of senescent mesenchymal stem cells 1) with classical SASP (oncogenic induced senescence (OIS)), 2) with non-classical SASP (Knock-out *RELA*) and 3) without non-classical SASP (knock-out *RAB27A* (sEV biogenesis pathway) using the shotgun technique Tandem Mass Tag (TMT) Systems (10-plex). **Results:** Comparative proteomic analysis identified 4099 proteins of which 25 were differentially regulated (11 down and 14 up) by non-classical SASP in comparison. The functional enrichment analysis indicated that metabolic pathways related to COPI-mediated anterograde transport, glycine degradation, Intra-Golgi traffic and retrograde transport at the Trans-Golgi-Network are involved in non-classical SASP.
Conclusion: For the first time, this study has provided evidence about the importance that Golgi apparatus´ traffic and transport are involved in the non-classical SASP. Altogether, these data collectively propose key that will be useful to design new therapeutic strategies in personalised medicine to increase their efficiency and stratifying patients to treat age-related diseases. **Funding:** JFL was funded by Proof-of-concept from ProteoRed-ISCIII (PPC2020) and Xunta de Galicia, Grant Number ED481D-2021-020. The proteomic analysis was performed in the Proteomics Unit of Complutense University of Madrid, a member of ProteoRed and is supported by grant PT17/0019, of the PE I+D+i 2013- 2016, funded by ISCIII and ERDF." Or "Grant PRB3 (IPT17/0019 - ISCIII-SGEFI / ER. MCA. received a grant from the Spanish National Health Institute Carlos III (PI20/00497).

S-nitrosoproteomics defines a key mechanism of exercise-induced cardioprotection

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Ischemic heart disease is the first leading cause of death worldwide [1]. Currently, reperfusion is the definitive treatment for acute ischemic heart disease [2]. Whereas regular physical activity is proven to help prevent and manage cardiac diseases, the mechanisms involved are not yet fully understood. Activation of the endothelial nitric oxide synthase (eNOS) by exercise training and its association with mitochondria were reported to strongly contribute to heart protection [3-5]. Thus, post-translational S-nitrosylation of protein cysteine residues has emerged as a possible key mechanism in NO-mediated cardioprotection.

In this study [6], proteomics was associated to cellular physiology experimentations to characterize the S-nitrosoproteome in mitochondria proteins from sedentary and exercise-trained rat hearts. Proteomics data were obtained using a cysteine-reactive tandem mass tag and enrichment procedure, followed shotgun proteomics by nano liquid chromatography tandem mass spectrometry identification. In response to exercise training increased NO bioavailability in mitochondria and S-nitrosylation of several key proteins involved in cardiac injury during ischemic reperfusion were found. Ex-vivo and in-vivo results together confirm that S-nitrosylation has a major role in mitochondrial ROS production and in regulation of calcium handling. Altogether the results provided evidence that cardioprotection by exercise training is explained by the re-localization of eNOS/NO/SNO signaling to mitochondria.

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Protein correlation profiling re-defines the protein composition of extracellular vesicles.

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The landscape of extracellular vesicles (EVs) types reported in literature is constantly increasing, including exosomes, microvesicles, large oncosomes, apoptotic bodies, exomeres and other nanoparticles. These vesicular entities possess key regulatory functions in cell-cell communication processes and are often classified on the basis of their size, composition and biological origin. The encapsulated protein content of EVs reflect their cell of origin and, consequently, EVs have a huge potential as biomarkers for cancer and other diseases. Over the years, many different approaches have been developed for the isolation of EVs. However, they possess inherent limitations and technical biases, resulting in heterogeneous preparations contaminated by other EVs subtypes, non-vesicular nanoparticles structures and even soluble proteins. This is especially problematic when specific regulatory functions are attributed to EVs subtypes. Therefore, a systematic characterization of the protein composition of the different types of EVs is needed.

Here, we report on the development of a proteomic strategy for the systematic characterization of the genuine protein content of small EVs. Our strategy involves a first purification of small EVs using differential ultracentrifugation and a second step using high-resolution density gradients. Owing to their low resolution, this two-step purification still yields sEVs fractions contaminated by significant amounts of non-vesicular proteins. To solve this limitation, all DG fractions are analysed by DIA and a proteome correlation profiling (PCP) approach is then applied to deconvolute the data and classify proteins displaying similar separation profiles across the density gradients. Using this strategy, we can unambiguously assign each protein to either vesicular or the non-vesicular populations with high selectivity. The application of this optimised method on a panel of cancer cell lines will generate a highly curated catalogue of pan-human and ubiquitous proteins present in these vesicular entities and will provide valuable information on the potential mechanisms of cargo selection.

Application of 2D DIGE to study the effect of ageing on the myofibrillar sub-proteome of horse meat

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Introduction. *Post-mortem* ageing practices are commonly applied by the meat industry to improve the quality of traded meat products. Ageing refers to the intervention by which meat is chill-stored under vacuum for a certain duration in order to develop desired quality attributes, especially an optimum degree of tenderness. *Post-mortem* tenderization of meat occurs by the action of endogenous muscle proteases that cause the degradation of structural proteins. This process has been scarcely investigated in horse meat, despite its gained popularity during recent years. Indeed, while beef is typically aged between 14 and 21 days, standardized ageing practices are lacking in the case of horse meat. In this regard, the objective of this study was to investigate the effect of ageing (0 *vs*. 21 d) in the myofibrillar sub-proteome of horse meat using 2D DIGE.

Materials and methods. *Longissimus thoracis et lumburum* muscle was excised from six Hispano-Bretón horses and two steaks (1.5 cm) from each muscle were cut, vacuum packed, randomly assigned to an ageing time (0 or 21 d) and aged at 4 ° C without illumination. Myofibrillar proteins were extracted and minimally labelled with CyDyes (GE Healthcare). Cy2 was used for the internal standard (a pool of all samples) whereas Cy3 and Cy5 were used for individual samples. 2D DIGE was carried out as previously described by Miller (2012) [1], applying commercial IPGs with pH 3-10 as a first and 10-15 % gradient gels for SDS-PAGE in the second dimension. Images were evaluated with DeCyder software (GE Healthcare) and spots with statistically significant differential abundance ($p \leq 0.05$) and sufficient intensity were excised from the DIGE gels after silver staining. Spots were then subjected to trypsin digestion and proteins identified by LC-MS/MS.

Results. 2D DIGE found 36 spots that were differentially abundant between 0 and 21 d aged samples. From them, 8 spots from structural proteins were identified as either intact proteins or protein fragments from muscle tissue. The decreased abundance of myosin binding protein C after 21 d ageing, together with the increase of three of its fragments (two of 110 kDa and one of 66 kDa) revealed, for the first time, that this protein is degraded during the ageing process of horse meat. The same was also observed for desmin, a key constituent of the intermediate filament in skeletal muscle, whose abundance decreased after 21 d of ageing. Actin was also degraded, visible as the formation of an aggregate and a fragment of actin in the gel. Interestingly, a fragment of troponin T, previously defined as an indicator of tenderness development in beef, was also identified in horse meat after three weeks of ageing. Overall, the present study represents a step forward in the understanding of the molecular mechanism of horse meat ageing by the application of gel-based proteomic techniques.

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Relationship between salivary proteome and xerostomia symptoms

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Saliva is a biological fluid composed by water, electrolytes and organic compounds with an important role in oral cavity lubrification and protection.

Xerostomia is characterized by a subjective dry mouth sensation, which can be due to a true hyposalivation or not. This pathology can be from diverse origins, being the most common autoimmune diseases, poly-medication, radiotherapy in the head and neck area or even idiopathic. Different symptoms are considered in xerostomia evaluation, including oral discomfort, difficulties in chewing, speaking and swallowing, among others, affecting well-being, food choices and diet. The most usual treatment is pilocarpine administration to relieve symptoms. This agonist of the parasympathetic nervous system induces fluid production. However, the total volume of saliva produced is variable among patients with xerostomia, suggesting that not only the amount of saliva may influence, but also the composition of this fluid.

The objective of the present study was to relate saliva protein profiles with xerostomia patients' symptoms. Besides protein profile, and since xerostomia can be related to changes in taste perception, the focus was given to two salivary proteins related to sweet and bitter tastes, namely amylase and cystatins, respectively.

Saliva samples were collected by the passive droll method to patients with xerostomia in a specialty appointment in Instituto de la Boca Seca, Barcelona (N=58). Collections were made in 2 different conditions: 1) at arrival, without stimulation; 2) 5 min after a pill of pilocarpine (*Salagen* 5mg). These samples were analyzed for pH, total protein concentration, and SDS-PAGE profile. Moreover, alfa-amylase enzymatic activity was determined. Western-blot was used to identify alpha-amylase and S-type cystatins.

Whereas pH and alpha-amylase activity were not observed to be statistically associated with xerostomia symptoms, an association was observed for some protein bands: a band identified as containing Ig polymeric receptor and proline-rich proteins were negatively associated with symptom levels. Amylase containing bands were also associated with symptoms, although Western-Blot analysis suggested that the association could be for other proteins in those bands (with similar molecular masses), rather than amylase. S-type cystatins were not related to xerostomia symptoms.

These results will be presented and this potential association of salivary proteome with xerostomia symptoms will be discussed, as well as further work needed to be done to deeper understand this pathology.

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CIRCULATING PROTEINS ASSOCIATED WITH RESPONSE AND RESISTANCE TO NEOADJUVANT CHEMOTHERAPY IN HER2-POSITIVE BREAST CANCER

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Abstract: Despite the increasing use of neoadjuvant chemotherapy (NAC) in HER2-positive breast cancer (BC) patients, the clinical problem of predicting individual treatment response remains unanswered. Furthermore, the use of ineffective chemotherapeutic regimens should be avoided. Serum biomarker levels are being studied more and more for their ability to predict therapy response and aid in the development of personalized treatment regimens. This study aims to identify effective protein networks and biomarkers to predict response to NAC in HER2-positive BC patients through an exhaustive large-scale LC-MS/MS-based qualitative and quantitative proteomic profiling of serum samples from responders and non-responders. Serum samples from HER2-positive BC patients were collected before NAC and were processed by three methods (with and without nanoparticles). The qualitative analysis revealed differences in the proteomic profiles between responders and non-responders, mainly in proteins implicated in the complement and coagulation cascades and apolipoproteins. Qualitative analysis confirmed that three proteins (AFM, SERPINA1, APOD) were correlated with NAC resistance. In this study, we show that serum biomarker profiles can predict treatment response and outcome in the neoadjuvant setting. If these findings are further developed, they will be of significant clinical utility in the design of treatment regimens for individual BC patients.

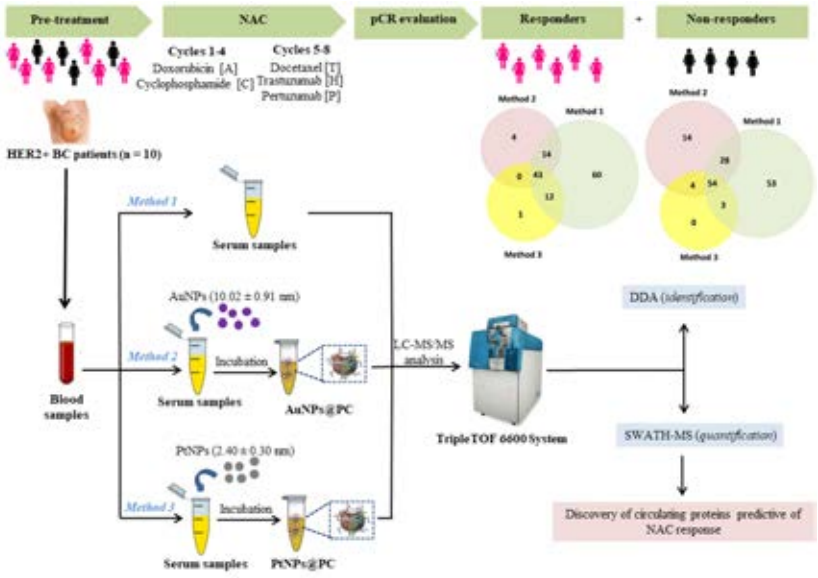

Figure 1. A schematic diagram of experimental workflow.

Extensive energy metabolism reprogramming and pathways of neurodegeneration induced by the Inhalation of ultrafine particulate matter revealed by label-free absolute quantitative proteomics

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The increasing amount of particulate matter released by combusted diesel vehicles, industrial emissions, and other anthropogenic sources has drawn the public's attention to its effects on health and quality of life. Several studies correlate this with an increased onset and mortality of pulmonary, cardiovascular, and neurodegenerative diseases. Although the cause-effect is known, the pathways that provoke neurodegeneration are not well established [1].

The experimental setting encompasses mice exposure to ultrafine particulate matter (UFPM) for one, three, and six months together with matching control groups for a total of six experimental conditions and a total of 36 mice (6 per experimental group). In this work, we used label-free and standard-free absolute protein quantification to gain insights into the proteome dynamics and their correlation to neurodegeneration.

Our approach has quantified 1738 proteins, from which 141 are statistically significant at one month, with 82 up-regulated and 59 down-regulated, 192 are statistically significant at three month, with 120 up-regulated and 72 down-regulated, 219 are statistically significant at six month, with 85 up-regulated and 134 down-regulated in the mice exposed to UFPM in comparison to the control group (Student's T-test with a 5% of permutation-based FDR filter).

Several statistically significant proteins participate in neurodegeneration-related pathways (KEGG Pathways of neurodegeneration), oxidative phosphorylation (OxPHOS), calcium signaling, WNT signaling, and synapsis. These findings suggest that the inhalation of UFPM is responsible for an extended dysregulation of energy metabolism and down-regulation of OxPHOS through a reduced capacity of complex I assembly (NDUFA11) [2] protein that is always reduced in the mice exposed to UFPM. The neuronal activity requires rapid adaptation of oxidative energy metabolism and a sufficient supply of oxygen and nutrients. Neuronal activity (NA) consumes significant amounts of ATP and stimulates ATP synthesis through a Ca^{2+}-dependent increase in OxPHOS. Thus, NA is very sensitive to altered mitochondrial function. Also, exposure to UFPM induces up-regulation of cellular response to hypoxia. This observation is compatible with airway inflammation caused by UFPM. Overall contributes to reduced synaptic capacity, leading to physical and cognitive impairment associated with neurodegeneration.

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Proteomics Analysis of ΔNp73 Effectors Identifies Proteins Associated with Lymphangiogenesis, Vasculogenesis and Metastasis in Colorectal Cancer

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Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer related death worldwide. Common events in CRC are p53-family alterations. Among the p53 family, ΔNp73 shows oncogenic properties but its effectors remain mostly unidentified.

In this work, we aimed to identify and functionally characterize the effectors of ΔNp73 by in-depth functional proteomics analysis of the secretome of human colorectal cancer cells HCT116. The secretome of HCT116 cells stably overexpressing ΔNp73 was analyzed and compared to the secretome of HCT116 Mock control cells using both high-density antibody microarrays and stable isotopic metabolic labeling (SILAC). In total, 493 chemokines, proangiogenic, and growth factors were surveyed for alteration by antibody microarrays, whereas by quantitative SILAC proteomics analysis 1051 proteins were identified and quantified in *forward* and *reverse* experiments. Data validation was performed by semiquantitative PCR, ELISA, dot-blot, and western blot. Evaluation of selected effectors as blood-based biomarkers in CRC was carried out using 60 plasma samples from CRC patients, colorectal individuals carrying premalignant lesions and colonoscopy-negative controls to validate their clinical potential.

A total of fifty-one dysregulated proteins by ΔNp73 with at least a 1.5 fold-change expression dysregulation were observed in the secretome of HCT 116 cells. We found three large protein-interacting clusters and an important association between the overexpression of ΔNp73 and effectors related to lymphangiogenesis, vasculogenesis and metastasis such as BDNF and the putative EMAP-II-VEGFC-VEGFR3 axis. In addition, we also demonstrated the usefulness of BDNF as a potential CRC biomarker to discriminate between CRC patients and premalignant individuals from controls with high sensitivity and specificity.

Mass-spectrometry based proteomics applied to the conservation of the Iberian lynx (*Lynx pardinus*)

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Chemical communication in mammals is mediated by olfactory signals deposited in the environment. Scent marks contain a mixture of molecules that convey information about sex, age, reproductive state and social status of the owners. Receptor animals modify their physiology and behaviour in response to such signals and initiate a learning process, which help them to recognize a social group, an individual or their relationship with the scent owner. Therefore, understanding chemical communication is essential for the conservation of a species since it plays an important role in mate selection, social organization and territorial maintenance.

The Iberian lynx (*Lynx pardinus*) is the most endangered feline species in the world. It was included in the Red List of threatened species at the end of the nineties, when a population survey revealed that fewer than a hundred individuals were left. This estimation led to the urgent design of an action plan directed at reversing the dramatic rate of loss of this species. The ex-situ programme has been very successful in increasing the number of individuals in the wild. However, our understanding of chemical communication in the Iberian lynx, a key aspect of its physiology, is still very scarce.

The aim of our project is to unravel the molecular basis of intra-specific communication in the Iberian lynx with the objective to exploit natural chemical signals to improve conservation programmes. In the present study, we show some pheromone candidates identified by using mass spectrometry to characterize volatile, protein and peptide composition of urinary sprays and facial marks. These molecules could be used to design more successful plans for the recovery and conservation of the Iberian lynx.

Association between calcific aortic valve disease and coronary artery disease: importance of the albumin redox state.

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Calcific aortic valve disease (CAVD) and coronary artery disease (CAD) are related cardiovascular pathologies in which common processes lead to the calcification of the corresponding affected tissue. Among the mechanisms involved in calcification, the oxidative stress that drives the oxidation of sulfur containing amino acids such as cysteines (Cys) is of particular interest. However, there are important differences between CAVD and CAD, particularly in terms of the reactive oxygen substances and enzymes involved.

To evaluate what effect that CAD has on aortic valves, we have performed a proteomic analysis using the FASILOX technique on valve tissue from patients with severe calcific aortic stenosis with and without CAD. This allowed us to quantify proteins and reversible Cys modifications simultaneously in the same experiment. We identified 16 proteins with different levels of expression between the two conditions studied. Besides, we found 7 peptides with differences in the redox state of their Cys. Importantly, we identified two specific sites of Cys oxidation in albumin that have not been described previously.
A functional analysis was, then, performed using the online tool DAVID, and based on this, 4 differentially expressed proteins of interest involved in cardiovascular processes were selected. These proteins were verified and quantified by two orthogonal techniques of high clinical potential such as Parallel Reaction Monitoring (PRM) and Western Blotting in plasma samples from an independent cohort of patients. Besides, protein albumin was selected for confirmation using the PEG-PCMal labelling technique, which allowed us to visualize changes in the redox state of this protein by electrophoretic analysis and immunodetection.

These results provide evidence that CAD affects valve calcification, modifying the molecular profile of aortic valve tissue. In addition, the study of the redox proteome has allowed us to define two new sites of Cys oxidation in albumin that have not been described previously, which represents a starting point for future functional studies.

Tailored proteomic pathway analysis for monitoring of bladder cancer patients

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T1 stage bladder cancer (BC) invades the bladder's lamina propria and, although sharing many genetic markers with muscle-invasive bladder cancers, is categorized as a non-muscle invasive tumor. On the other hand, it has a high risk of progression and requires precise surgery and regular costly and invasive endoscopic surveillance. Overall, patients with BC T1 stage have a significantly larger global burden and due to the high tendency for metastasis, a clinical decision for an aggressive treatment must be carefully handled [1,2].
To overcome this constant clinic dilemma, a unique concept named differential Personal Pathway index (dPPi) was developed to support healthcare decisions. The dPPi, combines the proteomic information on urine samples onto the biological pathways, enabling an easy, economical, and non-invasive follow-up of patients. Using a mass spectrometry-based personalized medicine approach, urinary proteomes of 30 patients pathological accessed and diagnosed with clear T1 stage bladder cancer were accessed and analysed using bioinformatics tools. Furthermore, the proteome changes of 6 patients were followed up using the dPPi algorithm during the disease course and medical interventions providing information to aid in clinical decision making.
Our results show that coupling the urine proteome quantification with the dPPi algorithm into a pathway quantification enables tracking the progression of BCa illness. Furthermore, this unique technique is utilized to follow up on patients and, in conjunction with clinic data, to aid in clinical decision making. This illustrates how proteomics open the way for individualized therapy and how pathway analysis may be used to support clinical healthcare decisions.

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Metabolic dyshomeostasis induced by SARS-CoV-2 structural proteins reveals immunological insights into viral olfactory interactions

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One of the most common symptoms in COVID-19 is a sudden loss of smell. SARS-CoV-2 has been detected in the olfactory bulb (OB) from animal models and sporadically in COVID-19 patients. To decipher the specific role over the SARS-CoV-2 proteome at olfactory level, we characterized the in-depth molecular imbalance induced by the expression of GFP-tagged SARS-CoV-2 structural proteins (M, N, E, S) on mouse OB cells. Transcriptomic and proteomic trajectories uncovered a widespread metabolic remodeling commonly converging in extracellular matrix organization, lipid metabolism and signaling by receptor tyrosine kinases. The molecular singularities and specific interactome expression modules were also characterized for each viral structural factor. The intracellular molecular imbalance induced by each SARS-CoV-2 structural protein was accompanied by differential activation dynamics in survival and immunological routes in parallel with a differentiated secretion profile of chemokines and growth factors in OB cells. Through a proteotranscriptomic data integration, a machine learning approach uncovered the TGF-beta and Hippo pathways as potential confluent activation nodes by the SARS-CoV-2 structural proteome. Taken together, these data provide important avenues for understanding the multifunctional immunomodulatory properties of SARS-CoV-2 M, N, S and E proteins beyond their intrinsic role in virion formation, deciphering mechanistic clues to the olfactory inflammation observed in COVID-19 patients.

Sex-dependent molecular changes in the olfactory tract in Alzheimer's and Parkinson's diseases

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Smell impairment is one of the earliest features in Alzheimer's (AD) and Parkinson's diseases (PD). However, the underlying molecular mechanisms associated to the olfactory dysfunction are poorly understood. We applied sequential window acquisition of all theoretical fragment ion spectra mass spectrometry (SWATH-MS) in 57 postmortem olfactory tracts (OT) derived from non-demented (n=6F/11M), AD (n=4F/13M) and PD (n=7F/16M) subjects. From the 1835 quantified proteins, around 11% varied between groups. Interestingly, 35 and 20 proteins were commonly deregulated across both sexes in AD and PD, respectively. Our preliminar data point out sex-dependent differences in terms of olfactory proteostasis, pathway modulation and protein networks. Our workflow is currently being complemented with secretability and neuropathological stage-dependent analysis in order to propose potential sex-specific fluid biomarkers and to increase our knowledge about the AD and PD progression at olfactory level in women and men.

Keywords: Alzheimer's disease, Olfactory tract, Parkinson's disease, Proteomics, Sexual dimorphism, SWATH-DIA, Systems biology.

Platelet lipidome fingerprint of obese patients: new assistance to characterize platelet dysfunction in obesity

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Obesity is associated with a pro-inflammatory and pro-thrombotic state that supports atherosclerosis progression and platelet hyperreactivity. During the last decade, the platelet lipidome has been considered a treasure trove as a source of biomarkers for preventing and treating different pathologies. The goal of the present study was to determine the lipid profile of platelets from severely obese patients. Twelve severely obese patients (BMI>40 kg/m^2) and their age- and sex-matched controls participated in the study. Platelets were isolated by an established method that limits contamination from other blood cells and plasma proteins. After that, lipids were extracted and major phospholipids, sphingolipids and neutral lipids were analyzed either by gas chromatography or by liquid chromatography coupled to mass spectrometry. Despite a significant increase in obese patient's plasma triglycerides, there was no significant differences on the membrane levels of triglycerides in platelets among the two groups. In contrast, total platelet membrane free cholesterol was significant decreased in the obese group. The profiling of phospholipids showed that phosphatidylcholine and phosphatidylethanolamine contents were significantly reduced in the obese group. However, no differences were found on the sphingomyelin and ceramides levels. The outline of glycerophospholipids and sphingolipids molecular species (fatty-acyl profiles) was similar in the two groups. As summary, this lipidomics data indicate a unique lipid fingerprint in platelets from obese patients. These results may guide further studies and provide mechanistic-driven perspectives related to the hyperactivate state of platelets in obesity.

Transposable Element-derived peptides are preferentially presented on HLA-A3 and HLA-A11 molecules

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Non-coding genomic regions are a relevant source of antigen presentation, and they have shown to be capable of generating anti-tumor T cell responses. A large fraction of the non-coding genome is composed of transposable elements (TEs), which include DNA transposons, as well as three main classes of retrotransposons (SINE, LINE and LTR). In Bonte PE and Arribas YA et al. (in revision), we combined scRNAseq and immunopeptidomics to identify TE-derived HLA-I peptides in glioblastoma tumors. Interestingly, we observe an enrichment for TE peptides presented on HLA-A3 and HLA-A11 molecules, while they are impaired on HLA-A2, HLA-B40 and HLA-B44. To understand if this preference is specific for TEs or corresponds to any non-canonical translation event, we used transcriptome assembly coupled to monoallelic immunopeptidomics from 25 different HLA-I molecules in B721.221 cell line. As result, we can observe that around 60% of transcripts overlap, as expected, protein-coding regions, while 10% are derived from TEs. MS/MS immunopeptidomics interrogated with *in silico* translated transcriptome shows that TE-peptides are enriched, when compared to total peptidome, in HLA-A3 and HLA-A11, in line with what we initially observed in glioblastoma. No preference for a particular HLA-I molecule is detected for the other non-canonical transcripts (lncRNA, ncRNA, pseudogenes, etc.), suggesting TE-specificity. A better detection of low abundant peptides in HLA-A3/A11 could be expected, because they generally end with lysine or arginine, resembling tryptic peptides. However, no enrichment is observed for HLA-A31/A33/A68 peptidomes, whose binding motif ends with arginine, discarding that more TEs are detected on HLA-A3/A11 because of technical basis. In addition, HLA-A3/A11 show a high enrichment for LTR, which are TE families with still some intact Open Reading Frames, because of their younger insertion in the genome. Together with the fact that HLA-A3/A11 presented TEs have lower expression levels than HLA-A2/B40/B44 presented ones, it suggests that young TE sequences are more permissively loaded in these HLA-I molecules. Which is the cause of this phenomenon? Are because differential amino acid proportions in more conserved LTR? The effects of the co-evolution between TEs and HLA-I on shaping TE sequence degeneration still need to be completely understood, since this could increase tumor immunogenicity for certain HLA-I peptide repertoires.

DeST: Deep Semantic Tagger

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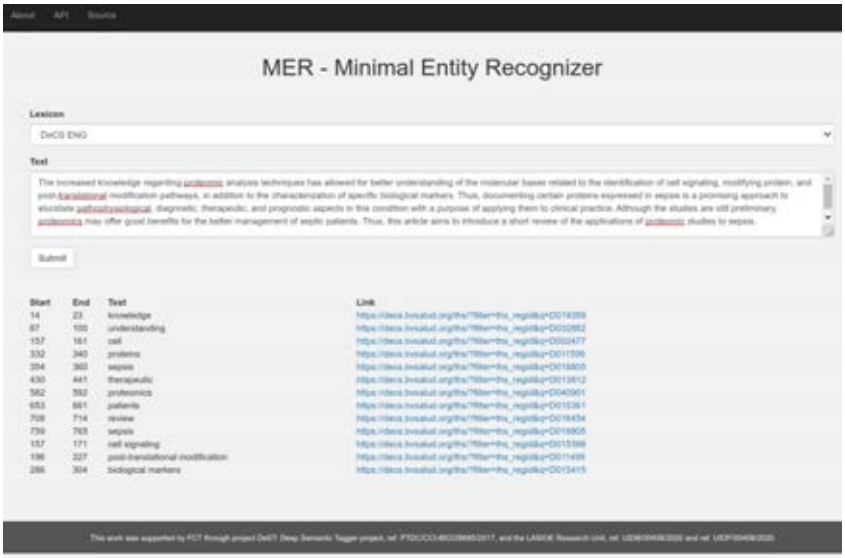
Besides the exponential growth of knowledge bases (KB) and the initiatives to connect them, most of our biomedical knowledge is still locked in free text. Free text has been and continues to be for humans the traditional and natural mean of representing and sharing knowledge. However, the knowledge encoded in free text hinders its accessibility and usage, since the retrieval of information from a large corpus is a tedious and time-consuming task for humans and a hard and prone to error task for machines. The ability to automatically process text enable us to more effectively navigate, retrieve information, find evidence, updates or even discern relevant from irrelevant information. Effectively linking text to KBs will also enhance the computer's ability to infer new knowledge. However, all these benefits require in-depth text mining solutions accessible to any researcher. With this goal in mind, the Deep Semantic Tagger (DeST) project developed open source software to perform the common tasks of a text mining pipeline, namely Named-Entity Recognition and Named-Entity Linking, Relation Extraction, and Semantic Similarity.



The performance of this software was extensively assessed in shared tasks using available corpora and datasets, with many of them achieving top-ranking positions in international challenges. Some of them are also available as web tools, such as the MER - Minimal Entity Recognizer (screenshot shown in the image). Given that most of these tools are based in machine learning techniques that require training data, the project also produced open access corpora and datasets that any researcher can use to enhance their models.

The project also published an open access book[1] that aims at helping Health and Life specialists or students to easily learn how to process data and text, by showing how shell scripting can help solve many of the data processing tasks that Health and Life specialists face everyday with minimal software dependencies.

The open source software, corpora and datasets are available at: https://github.com/lasigeBioTM; the web tools and all the book material at http://labs.rd.ciencias.ulisboa.pt/

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Finding biomarkers in thyroid cancer lesions: a mixomics approach

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Thyroid cancer is a common endocrine malignancy; however, its diagnosis is not straightforward. The current gold standard for diagnosing thyroid cancer is fine needle aspiration biopsy (FNAB), but when cytological analysis does not provide definitive results, in particular whether the lesion is benign or malignant, patients are often referred to diagnostic surgery. Given that not all these nodules require removal, nor all of them are malignant, this surgery would have been spared had the initial FNAB diagnosis been conclusive. There is therefore a need for more reliable and specific biomarkers for thyroid cancer malignancy at the FNAB stage that can complement and improve current diagnoses.

Proteomics by liquid chromatography-mass spectrometry (LC-MS) and metabolomics by high resolution-magic angle spinning nuclear magnetic resonance (HR-MAS NMR) and LC-MS were applied in thyroid nodules from patients with benign and malignant lesions. After collection at Centro Hospitalar de São João, samples were analysed intact by HR-MAS on a Bruker Avance III HD 500 MHz NMR spectrometer. Then, tissue was recovered and homogenised. First centrifugation removed cell debris, and after adding methanol 4 times the sample volume, a second centrifugation separated proteins (in the pellet) from metabolites (in the supernatant). Supernatant was evaporated and resuspended in 2% acetonitrile and 0.1% formic acid and analysed for LC-MS/MS metabolomics, while the pellet was prepared for Short GeLC [1], protein digestion by trypsin and peptide extraction for bottom-up proteomics on a TripleTOF™ 6600 Sciex® System.

A total of 3314 proteins were identified and quantified, while LC-MS untargeted metabolomics detected a total of 2347 monoisotopic features after filtering. By combining HR-MAS NMR with LC-MS metabolomics, a total of 46 metabolites were confidently identified. Differences between benign (adenomatous nodules) and malignant (differentiated thyroid cancer) were explored individually for each dataset and then combined in a mixomics approach. While proteomics and metabolomics each provided a list of interesting biomarkers, integration of both approaches yielded a biomarker panel with high sensitivity and specificity.

This work demonstrates the complementarity of NMR and mass spectrometry in metabolomics analysis, particularly in improving metabolite identification and differential diagnosis. Moreover, the combined proteomic and metabolomic data were consistent with high energetic and growth rates of cancer cells, and could potentially improve diagnosis at the FNAB stage.

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MASS SPECTROMETRY-BASED PROTEOMIC AND METABOLOMIC PROFILING OF SERUM SAMPLES FOR DISCOVERY AND VALIDATION OF TB DIAGNOSTIC BIOMARKERS

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Introduction: Tuberculosis (TB) is a transmissible disease listed as one of the 10 leading causes of death worldwide (10 million infected in 2019). A swift and precise diagnosis is essential to forestall its transmission, for which is crucial the discovery of effective diagnostic biomarkers.

Objective: Discover of molecular biomarkers for the early diagnosis of tuberculosis

Results: Two independent cohorts comprising 22 and 28 subjects were assayed by proteomics, and 44 were included for metabolomic analysis. All subjects were arranged into 2 experimental groups – healthy (H) and patients (P). LC-MS/MS protein and metabolite levels were submitted to multivariate, univariate and ROC analysis. An integrated ROC analysis was also performed for the 36 common individuals in the proteomic and metabolomic sets. From the 149 and 79 proteins identified in each set, 4 were found to be differentially abundant in both cohorts ($p>0.05$; $FC>\pm1.5$). The AUC, specificity and sensitivity determine by ROC statistical analysis for each proteomic set were 0.96; 86% and 100%; and 0.99; 100% and 85%. PLS-DA models created with the metabolites quantified in both modes: 69 (positive mode) and 32 (negative mode) allowed the discrimination between H and P. AUCs determined by ROC analysis comprising 5 metabolites for each mode were above 0.99 with all samples being correctly assigned to the respective experimental group. The determined parameters for the integrated ROC analysis enrolling the 14 elected biomarkers (AUC=1, specificity=100% and sensitivity=100%) and has correctly assigned the 8 individuals used only for prediction.

Conclusion: This multi-omics approach suggests 4 proteins and 10 metabolites as potential biomarkers for tuberculosis diagnosis. Two of the proteins are involved in antibacterial immune response. Validation of the proposed biomarkers require target analysis with a bigger cohort. Validated biomarkers are of potential use for the development of a point-of-care diagnosis clinical test.

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Spatial Proteomic Analysis of Isogenic Metastatic Colorectal Cancer Cells Reveals Key Dysregulated Proteins Associated with Lymph Node, Liver, and Lung Metastasis.

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Metastasis is the primary cause of colorectal cancer (CRC) death. Liver and lung are the most common sites of distal metastasis. We here aimed to study lymph nodes, liver, and lung CRC metastasis by quantitative multidimensional proteomics analysis (abundance and localization) using CRC cell models of these types of metastasis.

The isogenic KM12 cell system composed of the non-metastatic KM12C cells, liver metastatic KM12SM cells, and liver and lung metastatic KM12L4a cells, and the isogenic non-metastatic SW480 and lymph nodes metastatic SW620 cells, were used.

First, the secretome of the cells was collected before performing cellular fractionation into five subcellular fractions (cytoplasm, membrane, nucleus, chromatin-bound proteins, and cytoskeletal proteins) for their analysis by proteomics. Then, protein extracts of each subcellular fraction and the secretome were digested with trypsin and subsequently labeled with TMT 11-plex. In total, three TMT 11-plex were performed. Prior to LC-MS/MS analysis onto a Q Exactive, labeled peptides were fractionated using high pH reversed-phase peptide columns.

In total, we provide data on protein abundance and localization of 4710 proteins, and dysregulation of proteins in abundance and/or localization associated to the most common sites of CRC metastasis. After bioinformatics, alterations in abundance and localization for selected proteins were validated by orthogonal approaches (WB, IF, IHC, and ELISA) using CRC cells, and patient's tissue, and plasma.

Among the different results that supported the relevance of the proteomics analyses, it was particularly interesting the diagnostic ability of the measurement of GLG1 in plasma at advanced stages of the disease, and that the mislocalization of MUC5AC and BAIAP2 in the nucleus and membrane, respectively, was significantly associated with poor prognosis of CRC patients.

Keywords: colorectal cancer; metastasis; spatial proteomics; quantitative multidimensional proteomics; TMT; mass-spectrometry.

HUNTACYL: ALTERED DYNAMICS OF ACYLATIONS OF HISTONE H3 LYSINE 27 (H3K27): A MECHANISM CONTRIBUTING TO TRANSCRIPTIONAL DYSREGULATION IN HUNTINGTON'S DISEASE?

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Histones are proteins in charge of DNA packaging and compaction in eukaryotic nuclei. Histone post-translational modification (hPTM) describes the addition of a covalent chemical group to a specific histone residue in response to an environmental trigger. hPTMs thus serve as epigenetic knobs by which eukaryotic cells fine-tune DNA-templated processes such as replication, transcription and DNA damage repair. Dysregulation of hPTMs has been linked to disease states such as cancer and neurodegenerative disorders. Huntington's disease (HD) is a rare neurodegenerative disorder characterized by early aberrant transcription. Previous studies have revealed a decrease in acetylation levels at lysine 27 of histone H3 (H3K27ac) preferentially over gene bodies of neuron-identity genes in HD mice striatum, the brain region that is primarily affected in HD (1). Besides, over the past 15 years, structures which resemble acetylation but vary in length and hydrophobicity and can also modify lysines have been progressively discovered. They are collectively called acylations, and the big question that has emerged is whether they play specific roles compared to acetylation. Interestingly, H3K27 was recently reported to be modifiable by crotonylation in the brain. Besides, lactate is a key metabolite in this organ and was described in 2019 to induce histone lysine lactylation. Furthermore, new mouse H3 sequence variants have been discovered and are shown to have a role in regulating tissue-specific gene expression (2). Therefore, it is tempting to further explore their functional implication vis-à-vis disease phenotypes.

In our current project, we aim at deciphering the contribution of acyl-Lys PTMs in driving the early transcriptional reprogramming in HD as compared to lysine acetylation. To our knowledge, acylation marks have not been studied in the context of HD as of yet. However, screening for many combinations of biologically relevant lysine acylations and methylations on several H3 variants leads to numerous isobaric and isomeric tryptic peptides. Those peptidoforms can be difficult to resolve chromatographically or to distinguish by MS/MS using the traditional data-dependent acquisition (DDA) approach.

To address this issue, we are using targeted proteomics to detect and quantitate a specific list of hPTM combinations/variants with high sensitivity and quantitative accuracy. Practically, we envision utilizing parallel reaction monitoring (PRM) to identify and quantify variably modified H3K27acyl in canonical H3, H3.3, and two other mouse-specific H3 variants (H3mm7 and H3mm13) purified from brain tissues of normal and HD mice. In all, we expect that neuroepiproteomics will help elucidate the epigenetic disruption underlying HD pathomechanism and identify potential therapeutic targets.

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PROTEOMIC AND PHOSPHOPROTEOMIC CHARACTERIZATION OF MOLECULAR MECHANISMS UNLERLYING PROGRESSIVE FAMILIAR INTRAHEPATIC CHOLESTASIS TYPE 3

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Introduction

Progressive familiar intrahepatic cholestasis type 3 (PFIC3) is a severe rare liver disease which affects between 1/50,000 to 1/100,000 children[1]. In physiological conditions, bile is produced by the liver and stored in the gallbladder, then it flows to the small intestine to play its role in fat digestion. To prevent tissue damage, bile acids are kept into fatty acids micelles. In cholestatic situations this bile flow is disrupted. Mutations in phosphatidyl choline transporter ABCB4 (*MDR3*)[2] lead to intrahepatic accumulation of free bile acids that results in liver damage since early ages. Currently, the only treatment of this disease is liver transplantation[3].

Materials and methods

We performed a quantitative proteomic and phosphoproteomic analysis of 8 samples from control and PFIC3 patients. We have extracted and digested with trypsin proteins from liver tissue and then we have performed an isobaric labelled based quantification using TMT11plex. We separated the peptides in 10 fractions that where analyzed independently by LC-MS in an Orbitrap Exploris 240 (Thermo). Moreover, we performed a phosphopeptide purification with TiO2 beads to carry out a phosphoproteomic analysis. For data analysis we used Proteome Discoverer software for protein identification and quantification, and Ingenuity Pathway Analysis (IPA) for functional analysis.

Results and conclusions

We identified 56,345 peptides corresponding to 6,246 protein groups. 324 proteins showed differential expression between control and PFIC3 conditions (t-test: adj p value<0,05). Regarding phosphoproteomic analysis, we identified 1,964 proteins, 5,731 peptides and 5,090 phosphopeptides. 215 peptides were differentially phosphorylated in PFIC. IPA analysis revealed that canonical pathways including lipid metabolism, inflammation, cell survival and cytoskeleton organization were affected in PFIC3.

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Proteomics analysis of gastric cancer patients with diabetes mellitus

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Gastric cancer (GC) is the fifth most frequent cancer and the third leading cause of cancer deaths worldwide. Diabetes mellitus (DM) is also a major cause of death in the world and is known to be associated with an increased risk for several cancer types, including hepatocellular, pancreatic and urothelial carcinoma. A possible relationship between DM and GC has been discussed for several years due to their common characteristics, including hyperinsulinemia, hyperglycemia, and inflammation. In this study, we performed a comprehensive proteomics approach on GC samples from 40 patients aiming to elucidate the possible links between DM and GC. Upregulated proteins in the GC samples from diabetic patients were particularly enriched in respiratory electron transport and alcohol metabolic biological processes, while downregulated proteins were associated with epithelial cancers, intestinal diseases, and cell–cell junction cellular components. Taken together, these results support the data already obtained by previous studies that associate diabetes with metabolic disorders and diabetes-associated diseases, such as Alzheimer's and Parkinson's.

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Poster Session I

SHOTGUN PROTEOMICS OF RED BLOOD CELLS FROM OBSTRUCTIVE SLEEP APNEA PATIENTS UNDER POSITIVE AIRWAY PRESSURE (PAP) TREATMENT

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Obstructive Sleep Apnea (OSA) syndrome is characterized by recurrent episodes of apneas and hypopneas during sleep, leading to recurrent intermittent hypoxia and sleep fragmentation. No treated OSA can result in metabolic and cardiovascular diseases. By 2D gel-based proteomics approach we have demonstrated that OSA can cause alterations in the red blood cells (RBC) proteome that may be associated with OSA outcomes [1,2]. OSA induces alterations in the redox/oligomeric states of RBC proteins such as gyceraldehyde-3-phosphate dehydrogenase (GAPDH) and peroxiredoxin-2 (PRDX2) that can be reverted or modulated by PAP treatment [3].
In this study, we applied a shotgun proteomics strategy to further investigate the RBC proteome from patients with OSA before and after PAP treatment to better understand the regulation of RBC homeostasis in the context of OSA and/or under effect of PAP treatment.
As a first approach, RBCs samples, corresponding to Snorers patients as control (n=23) and patients with OSA before and after six months of PAP treatment (n=33/condition) were selected from our biobank[1]. Samples were randomly pooled (n=3 per group/condition) and lysed 1:6 with 5mM sodium phosphate buffer containing 100 mM of N-ethylmaleimide, a reagent that alkylates free sulfhydryl groups, before haemoglobin depletion by using Hemovoid^{TM} system. Depleted samples were alkylated, reduced and digested with trypsin and chymotrypsin. The resulting peptides were cleaned with C18 columns and analysed in triplicate by a Nano High Performance Liquid Chromatography (nanoHPLC) on-line coupled to a high-resolution accurate-mass Orbitrap mass spectrometer (Q Exactive, Thermo Scientific) with a nano electrospray ionization source (nanoESI). The acquired mass spectrometry data were analysed by MaxQuant v1.5.8.3 and Perseus v2.0.3.1 software.
The preliminary results corroborated our previous findings by showing that proteins associated with stress response and antioxidant regulatory system were the most changed in OSA RBC compared with Snorers ones. The active catalytic cysteine (Cys 51) in the PRDX2 was identified trioxidized $–SO_3H$ almost exclusively in OSA RBC before PAP treatment. Further analyses and validation of these data are in progress, which will certainly provide a better understanding of RBC molecular mechanisms and their proteins/PTMs associated with OSA pathology and/or response to PAP therapy.

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I.2

Investigating the impact of COVID-19 vaccines on the red blood cell immune function by omics-based approaches.

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The role of red blood cells (RBC) in the immune system is increasingly recognized. However, RBC-derived molecules with an immunomodulatory role in health and disease, as well as in vaccine immunogenicity are still poorly investigated [1,2]. Taking as a model the emergent COVID-19 vaccines, we aimed to investigate whether vaccines induce proteome and/or metabolome changes in RBCs able to affect T-cell immune activity, as a mechanistic test for vaccine immunization regulated by RBCs. Our ultimate goal is to identify RBC immunomodulators as potential co-adjuvants in the formulation of next-generation vaccines with bolstered efficacy and duration.

A biobank of blood samples collected longitudinally under ‘omics’ quality control from subjects (n=39) that underwent vaccination for COVID-19 between April and September 2021 was created. This biobank is associated with extensive clinical data, including demographic data, COVID-19 PCR diagnosis, hematological and vaccine effectivity data.

Linear Mixed Models, were used to evaluate the association between biometrical characteristics, health related habits, vaccine technology and vaccine effectivity and hematological parameters, along the different time-points (t0-t4) under study, i.e, before and after (24-72h or 30 days) of the first and second dose of vaccine. Statistical analyses were performed using R software version 4.1.2. Results showed significant differences ($p<0.05$) before/after vaccination in a set of hematological variables (e.g., hemoglobin, lymphocytes and monocytes values), as well in terms of vaccine effectivity and vaccine technology (mRNA or adenovirus – based vaccines).

Preliminary data from proteomics and metabolomics analysis of RBCs along the different timepoints (t0-4) of immunization response will be also presented and discussed.

The knowledge gained with this project can generate important evidence-based recommendations intended to optimize vaccine immunization, by recognizing the impact of blood cells such RBCs in the immune system regulation.

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Project approved by the Ethical Committee of INSA.I.P.-Lisboa and Centro Nacional e Proteção de Dados, Portugal.

OCCUPATIONAL SECONDHAND SMOKE EXPOSURE - A PROTEOMIC ANALYSIS

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WHO have stated that near 900 000 deaths per year result from Secondhand Smoke (SHS) exposure. Non-smokers exposed to SHS are at risk to develop tobacco smoke associated diseases and improved risk prediction and understanding of SHS-induced pathogenesis are needed.
We have previously shown that non-smokers workers from a group of Lisbon smoking restaurants presented higher levels of urinary cotinine and changes in their plasma proteome that might be associated with SHS exposure [1]. Herein, to better investigate the molecular biology involved in the cellular response to the SHS, we performed a proteomic study at the upper respiratory level of those occupationally exposed subjects.

In total, 25 Lisbon restaurants agreed to participate. Nasal epithelium and urine samples were collected from their employees (n=52) for proteomics analysis and cotinine evaluation of SHS exposure, respectively. The subjects were classified as never smoker (N), former smoker (F) and smoker (S); exposed (NE=11; FE=10; SE=4) or non-exposed (N=11; F=8; S=8) to SHS. All subjects were healthy and showed no significant differences in parameters like age, time in the workplace, tobacco smoking habits and spirometry evaluation of pulmonary function. Urine cotinine levels showed significantly elevated in the exposed subjects compared to non-exposed, confirming SHS exposure. Nasal epithelium samples were analyzed by shotgun proteomics using an ESI-LTQ-Orbitrap mass spectrometer. The obtained MS data was analyzed by the “PatternLab” software for protein identification. The identified proteins were submitted to the "ClueGO" application of the "Cytoscape" software, for functional annotation & enrichment analyses.

The results indicated that in NE subjects the SHS is associated with the biologic terms of “Lactate dehydrogenase complex” and “Pentose-Phosphatase Shunt”, also with “Glutathione peroxidase activity” and “T- cell apoptotic process”. At the other end the FE subjects presented a specific proteome enriched in biologic information with terms as the “L-Lactate dehydrogenase complex” and the “Peroxisome”. These data suggested that hypoxia and detoxification process seem to be activated in both NE and FE subjects in response to SHS exposure. Biological terms such as “Peripheral T cell lymphoma”, “Central carbon metabolism in cancer”, “Myelodysplastic syndrome”, “Monocyte & Granulocyte & Macrophage & Leukocyte Chemotaxis”, Nucleossome, variant H3.1-H2A2-H2B.1&Others”, and “DNA replication-dependent chromatin assembly” were also identified in FE subjects.
Altogether, these data indicated that the nasal epithelium proteome modulates in response to SHS exposure and cumulative and different biologic processes may be taking place in FE subjects, possibly due to their previous smoking habits, when compared to NE subjects. Further validation studies are needed to the better understanding the SHS exposure-induced mechanisms as risk factors for airway diseases.

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Endothelial Colony forming Cells (ECFCs) proteomic response to the serum factors of COVID19 asymptomatic or critical patients

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The virus responsible of Coronavirus-19 (COVID-19) disease, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), compromises the cardiovascular system causing vascular damage and thromboembolic events in critical COVID-19 patients, deriving in many related deaths and long-hauler symptoms. Understanding how these processes are triggered as well as the potential long-term sequelae, even in asymptomatic individuals, becomes essential.

Herein, we have evaluated, by application of a proteomics-based quantitative approach, the effect of serum from COVID-19 asymptomatic individuals and critical patients over Endothelial Colony Forming Cells (ECFCs), given the regenerative role of these cells and their potential role in COVID-19 disease. Thus, ECFCs were incubated *ex-vivo* for 24 hours with the serum of either COVID-19 negative (PCR-/IgG-, n:8), COVID-19 positive asymptomatic donors, at different infective stages: PCR+/ IgG- (n:8) and PCR-/IgG+ (n:8), or hospitalized, critical patients (n:8).

A label free quantitative (LFQ) approach was applied, with a TIMS-TOF Pro instrument (Bruker Daltonics) operated in data dependent acquisition (DDA) mode. Raw files were processed with MaxQuant and then loaded in Perseus for further statistical analysis.

Proteomics-based quantitative approach allowed the identification and further quantification of 5052 proteins (up- and down-regulated proteins depending on the conditions tested). Machine learning algorithms confirmed the discriminating potential of the proteins differentially expressed between the three groups. Besides, functional classification reported that proteins differentially expressed correlated with an inflammatory response after viral infection, RNA metabolism and autophagy, among others.

I.5

SWATH-MS REVEALS NEW INSIGHTS IN THE CELLULAR PATHOPHYSIOLOGY OF HUMAN HEART FAILURE WITH PRESERVED EJECTION FRACTION (HFpEF)

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Heart Failure with preserved ejection fraction (HFpEF) is a major health challenge affecting millions of people worldwide. HFpEF underlying cellular pathophysiological mechanisms remain largely uncharacterized. Moreover, HFpEF presents multiple etiologies and associated co-morbidities, leading to difficulties in diagnosis and limited therapeutic options.

This study employed a quantitative proteomic analysis by advanced mass spectrometry (SWATH-MS) to investigate signaling pathways and mechanisms that correlate with HFpEF. Protein expression profiles were analyzed in HFpEF and non-HFpEF human left ventricular myocardium biopsy samples. Functional analysis revealed several proteins that correlated with HFpEF, including proteins associated with mitochondrial dysfunction, oxidative stress reaction, and inflammation. Data obtained also reflects the heterogeneity of human heart biopsies and HFpEF clinical variables.

The proteomic characterization described in this work provides new insights and raises new questions related to HFpEF cellular pathophysiology, enabling additional studies focused on the development of novel therapies and diagnosis strategies for HFpEF patients.

Keywords: Human heart Failure; Heart Failure with preserved ejection fraction; Proteomics

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I.6

Stiffness and oxygen levels modulate common pathways in cultured mesenchymal stem cells - New tools for HIE treatment

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Hypoxic-ischemic encephalopathy (HIE) is a neonatal brain injury and is associated with a high mortality rate or permanent neurophysiological sequelae. This condition is caused by an episode of perinatal asphyxia that leads to the insufficient blood supply to the brain resulting in neural death. Therapeutic hypothermia is the most common clinical practice but is not as effective as initially thought. Mesenchymal stem cells (MSCs) are emerging as a promising therapeutic approach. Nevertheless, a human intravenous dose requires a very high number of cells considering their availability (2 million/kg). Additionally, recent studies suggest that extensive in vitro expansion may compromise MSCs stemness. Therefore, there is the need to develop innovative strategies to improve their potential. In this study, MSCs were cultured on platforms with controlled stiffness – mechanomodulation (≈3kPa) – or oxygen levels – physoxia (≈5%O_2) – to mimic a more physiological environment. Then, a proteomic screening of primed and "standard" (plastic (≈1GPa), at ≈18%O_2) expanded MSCs was performed, and proteins were quantified using DIA (SWATH-MS). For each condition, a PLS-DA model identified proteins with a VIP score above 1. Comparing the hits, a total of 204 proteins were common between the two conditions. A Gene Ontology analysis showed common modulated pathways, such as chaperonin (TRiC) or RNA polymerase I associated proteins. In addition, a deeper analysis allowed the identification of common downregulated processes, such as translational events or RNA metabolism. Interestingly, some upregulated pathways seemed to be associated with exosomes or mitochondria. Also, data analysis allowed the identification of JUN and FOS as the common transcription factors that regulate these overexpressed proteins. In conclusion, these screenings identified potential pathways shared by mechanomodulation and physoxia stimulus. In the future, we aim to explore these mechanisms and validate their relevance in improving stem cell therapeutic potential.

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I.7

Identification of large biomolecules as biomarkers of population lifestyle and industrial activities using environmental proteomics in wastewater-based epidemiology (EP-WBE).

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Many peptides and proteins constitute important biomarkers in clinical practice. We demonstrated using proteomic approaches, that wastewater taken at a wastewater treatment plant (WWTP) contains a huge range of proteins from different species, including human, known as disease biomarkers [1]. Thus, information carried by peptides and proteins in wastewater can be of high value both for wastewater-based epidemiology (WBE) monitoring and environmental status and human activity [2]. Despite the importance of these biomolecules, the protein composition of wastewater was practically unknown until now. On this basis, we have studied the differences of wastewater protein composition in 10 WWTP in Catalonia which serve communities of different population and industrial activity.

Up to 100 mL of 24-h composite wastewater samples were processed. Samples were filtered and the soluble part was concentrated in a SDS-PAGE gel. Gel slides were digested with trypsin and the resulting peptides analyzed by MALDI-TOF-MS and by HR-LC-MS/MS. Database search were done using the complete Uniprot database through the Proteome Discoverer software and a semiquantitative analysis was performed based on the area of the unique detected peptides.

We have been able to identify hundreds of proteins of human origin and other species. Some of the identified human proteins, such as uromodulin, α-amylase and S100A8, are well-known molecules proposed as health biomarkers. The analysis of the protein profile of other specie (rat, mouse, pigs, cattle and poultry) has allowed us to find markers of industrial activity in the different areas studied (Fig 1). These proteins can be a good indicator of the health status of the population and they also reflect the types of human activity (industry, agriculture and livestock).

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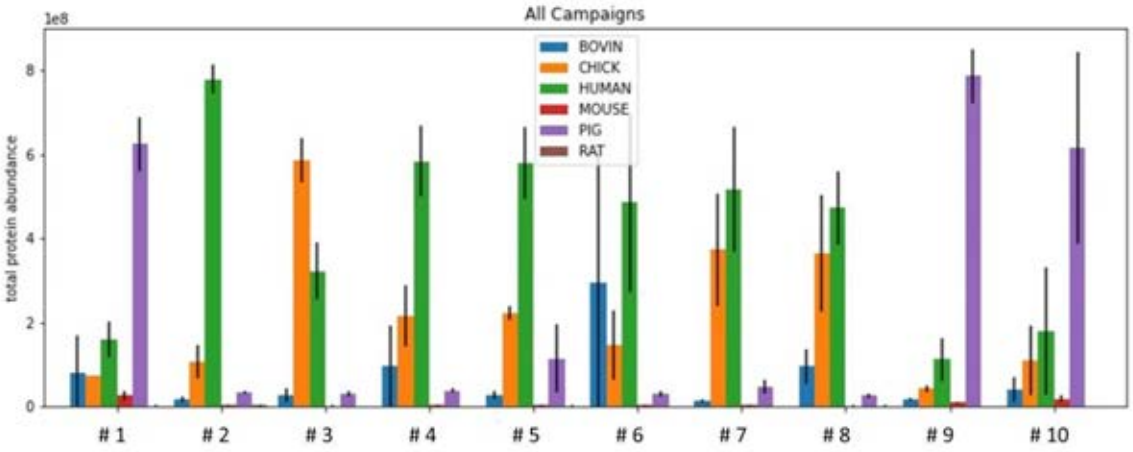
Figure 1



Proteome profiling of formalin-fixed paraffin-embedded (FFPE) tissue from stained histopathology glass slides.

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Massive amounts of FFPE tissue blocks are stored at the Pathological Anatomy Departments of Hospitals worldwide. Only in our site -the University Hospital of a Coruña (HUAC)- there are more than 1 million, and this number is rapidly rising, adding 12.800 new blocks so far this year. In this work, we describe a MS-based proteomic workflow for the qualitative profiling of FFPE tissues directly from their mold (cassette) and also from pre-stained histopathology glass slides. We have analyzed six synovial membrane tissues obtained from 3 rheumatoid (RA) and 3 psoriatic arthritis (PSA) patients. These samples belong to the HUAC Rheumatology Collection. One slice of 10um thickness was cut from each block and collected in 2mL microcentrifuge tubes. In parallel, two slices of 3um thickness from the same blocks were deposited onto glass slides and used for H&E histology. Both types of FFPE samples were deparaffinized and rehydrated. Proteins were extracted with lysis buffer by sonication/heat treatment and cleaned up by acetone precipitation. Protein pellets were air dried and re-suspended. Proteins content was determined by Bradford assay. Total amount of each sample was reduced, alkylated and digested with trypsin. Tryptic peptides were desalted via StageTips. 200ng of each peptide mixture were injected on a nanoElute LC coupled to a high-resolution TIMS-TOF. Peptides were analyzed in DDA mode with PASEF enabled. MS raw files were processed with PEAKS Studio 10.6.
We worked with two different types of FFPE sections to test varying tissue amounts and storage conditions. As expected, we found a higher protein yield in cassette samples (1- 19,7ug) compared to the glass slides (≤1ug). The sample with the highest number of proteins and peptides identified, respectively 3076 and 19356, was PSA_1 in cassette format. Globally, in slide format, we found a 2-fold higher PG mean value in PSA samples compared to RA ones. Among the samples extracted directly from the block, we didn't observe any statistical difference in term of number of PG identified between pathologies. Among PSA samples, we found a 2-fold higher PG mean value in cassette (1895) compared to slide (1076), whereas in RA samples we found a 5-fold higher value in cassette (2262) compared to slide (463). Only in one case (PSA_2), the number of PG was quite similar in both matrix: 1641 PG in cassette and 1977 in slide. Of the 2452 PG identified in total (1613 detected with more than 1 peptide) 78% were found in both samples, indicating that, at least in this case, protein extraction was highly comparable despite the noticeable differences in tissue format.
In this study, we illustrate the usefulness of different types of FFPE stored tissues for rheumatic disease phenotyping and patient stratification by means of proteomics, and we describe a protocol for the use of samples stored in glass slides after histological analysis.

Label-free quantitative proteomics analysis reveals potential diagnostic biomarkers associated with osteoarthritis severity in human synovial fluid.

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Introduction: Limitations on early diagnosis and effective pharmacotherapy for osteoarthritis (OA) are predominantly attributed to the current limited understanding of its pathogenesis. Pathological changes in the joint are expected to be represented by synovial fluid (SF) proteins, which are altered due to the disease and have significant biomarker potential. In this study, a proteomic analysis based on label-free quantification (LFQ) has been performed to explore the protein profiles of SF from different grades of cartilage damage in OA and healthy controls.
Methods: Post-mortem SF samples (n=61) from knee joints were used. Joints were graded based on the severity of changes in the knee cartilage surfaces using the Outerbridge scoring system, which grades joints from grade 0, control (n=5), early OA (n=24), middle OA (n=21) and late OA (n=11). Technical replicates of protein digest of SF proteins were analyzed by LC/MS/MS on a nanoElute-LC coupled to a high-resolution TIMS-QTOF (timsTOF Pro, Bruker Daltonics). Protein identification and quantification were performed using MaxQuant. Label free quantitation (LFQ) intensity data were used for further analysis and comparisons across the variant groups. Descriptive data analysis was performed using Perseus software. Statistical analyses were performed on R 4.1.3 software. Robust feature selection was carried out using Jonckheere-Terpstra (JT) non parametric statistic rank-based distribution-free. The Benjamini-Hochberg procedure was used to adjust p-values for Multiple Comparisons.
Results: 1,221 protein groups were identified in the synovial fluid samples. Among them, 979 protein groups were identified with more than 2 peptides, out of which 409 were quantifiable in all the technical replicates and were present in 70% of the samples within each experimental group. The Jonckheere-Terpstra (JT) test was used to select the best candidates by calculating the increase or decrease label-free quantitation Intensity (LFQ) through comparing the expression pattern of the proteins across the evolution of cartilage damage. 78 proteins, present in all the SF samples, were found with a trend to be increased in early stages of the disease compared to middle and late OA stages ($p<0.05$). Among these, 12 proteins were identified to be the most differentially expressed proteins ($p<0.01$). Validation of these potential OA diagnostic biomarkers is currently being carried out using immunoassays to confirm these exploratory results.
Conclusions: This study has identified a distinct protein profile in synovial fluids from individuals with different degrees of cartilage damage and healthy donors. It also reports potential clinically useful protein candidate biomarkers for OA diagnosis, which are associated with disease occurrence and may predict progression of cartilage degradation in OA.

Unveiling Parkinson's disease through discovery metabolomics

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Parkinson's disease (PD) is the second most common age associated neurodegenerative disorder affecting approximately 2% of the worldwide human population with age of 65 and above with tendency to increase the incidence. Yet, PD is the disease which mechanism is not fully understood, especially in idiopathic PDs, without reliable definitive and differential diagnosis in early onset and without cure. Current treatments are only symptomatic. In this work we studied plasma metabolome of the patients with PD aiming to identify key mechanisms and pathways of the disease, identify putative biomarkers and explore possibility to differentiate stages using untargeted metabolomics. Samples are obtained from the Biobanco-iMM CAML, Lisbon Academic Medical Center, Portugal and approved by the Ethical Committee for use in this study. Training set of samples was consisted of 25 samples of which 9 was healthy controls and16 of PD patients pooled according to HY stage defined by medical examination (Hoehn and Yahr scale). Upon deproteinization step, metabolic extracts were analyzed by tandem ultra-high resolution mass spectrometry LC-QTOF MS (Impact II, Bruker). Analysis was performed in both, positive (ESI +) and negative (ESI -) ionization mode. Acquity ULPC HSS T3 column was used for chromatographic separation. Internal calibration with sodium-format adducts was used for calibration of MS spectra. Raw Mass Spectrometric data was preprocessed (peak extraction, filtering, and alignment) and analyzed using Metaboanalyst 5.0 and Metaboscape 4.0 (Bruker). In total, it was detected 4052 and 1571 features in positive and negative mode respectively. Non-supervised (PCA and hierarchical clustering) and supervised classification was performed revealing statistically significant grouping of samples according to disease stage. Cross validation of PLS-DA revealed good prediction power with Q2 and R2 values of 0.86 and 0.99 respectively. Pathway enrichment analysis indicate over representation of molecules involved in biosynthesis of unsaturated fatty acids, linoleic acid metabolism and primary bile acid biosynthesis as a metabolic disorder involved in Parkinson´s disease. In conclusion, untargeted metabolomics of plasma applied to PD study showed to be promising tool for study of the disease mechanisms and that metabolic changes corelate with the severity of the disease. Future work includes extensive validation studies.

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Poster Session II

Serum proteomic profile of asthmatic patients after six months of benralizumab and mepolizumab treatment

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Severe eosinophilic asthma (SEA) is typically characterized by chronic airway inflammation, oxidative stress and elevated proinflammatory cytokines, specifically IL-5. Benralizumab and mepolizumab are two humanized monoclonal N-glycosylated IgG1/k antibodies directed against IL-5 receptor and IL-5, respectively, approved for SEA control. In order to investigate about optimal treatment duration, persistence of effectiveness and safety, we had previously conducted a proteomic analysis of SEA serum samples before (T0) and after 1 month of mepolizumab (T1M) and benralizumab (T1B) treatment, with interesting results [1-3]. Here we performed a further differential proteomic analysis introducing also SEA sera after 6 months of both therapies (T6M and T6B) and sera from healthy patients (CTRL). Identified proteins were used to perform bioinformatic analysis by MetaCore software and the proteins of interest were validated by immunoblot.

Proteomic analysis highlighted 82 differences among the six conditions, in particular, 30 referred to benralizumab timepoints (T0, T1B, T6B) and 24 to mepolizumab timepoints (T0, T1M, T6M) analyses. T-SNE and heatmap analysis showed that T0 and T1 samples were influenced by the different treatments, on the contrary all T6 samples were more similar to that of the healthy subjects, regardless of treatments. We validated differential proteins abundance by immunoblotting analysis, obtaining an increased level of plasminogen and ceruloplasmin after six months of both treatments. Moreover, APOAI, APOC-II and APOC-III were up-regulated principally after six months of benralizumab treatment, and negatively correlated with peripheral eosinophilia (cell/mm3 %). Enrichment analysis also suggested that the identified proteins were related to lipid metabolism and transport, blood coagulation and ECM remodelling.

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AUTOMATED WORKFLOWS FOR DIA DATA USING DIA-NN ON THE PASER PLATFORM

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Introduction

Data independent acquisition has become the go to method for deep and quantitative proteomic analysis given the ability to sample large m/z windows in a reproducible and non-stochastic manner. Using a method termed dia-PASEF on a TIMS enabled Q-TOF lends additional advantages in both duty cycle and selectivity using the ion mobility space. Dia-PASEF allows for deep proteomes in short gradient times (<20 min.) and therefore 100's of LCMS runs can be generated in short times. Data analysis in a streamlined automated fashion expedites the time from experiments to discovery. DIA-NN is a novel software package that uses neural networks providing best-in-class DIA output. Here we integrate DIA-NN onto the PaSER platform for a streamlined workflow for the analysis of many samples in a short analysis time with no file transfer or data migration.

Methods

A timsTOF Pro using variable CE's and mobility windows in gradients ranging from 5 min. to 90 min. were used. DIA-NN was modified to become CCS-enabled and process data in the most expedient fashion. The PaSER GUI was designed such that first-pass analysis is predefined automatically triggering quantitative analysis. Retrospectively, match-between-runs (MBR) analysis can be triggered on the whole project or subset of user defined experiments.

Preliminary Data

Human, Yeast and E. coli (HYE) digested mixtures at different but known ratios with injection loads from 50ng to 600ng were run at different gradient lengths in replicate resulting in >2500 proteins at short gradients to >9000 proteins identified and quantified at longer gradients. Quantitative accuracy was shown to be <20% CV. Using the DIA-NN as integrated into PaSER creates a seamless approach to dia-PASEF analysis.

Label-free single cell analysis workflow on the timsTOF SCP mass spectrometer using the CellenONE platform

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Introduction

Single cell proteomics is a rapidly developing field with the potential to make important contributions to the understanding of cellular heterogeneity. Recent enhancements in trapped ion mobility spectrometry (TIMS) coupled to fast and sensitive mass spectrometry established in the timsTOF SCP paired with automated single cell sorting and sample preparation realized with the cellenONE platform allows for sensitive proteome analyses at the single cell level. Coupled to developments in processing of data independent acquisition (DIA) mode data files using deep learning with neuronal networks (e.g. DIA-NN) further improves detectability and quantifiability of proteins from minimal input samples such as single cells.

Methods

20, 10, 5, 2 and 1 HEK 293 and HeLa cells were sorted, lysed, and digested using the cellenONE®, a liquid-dispensing instrument for cell isolation and picolitre dispensing. After single cell deposition and reagent dispensing, samples were directly incubated at 50°C with high humidity on deck of the instrument. Tryptic peptides were transferred into low protein binding autosampler vials and injected onto a 250 cm x 75 µm Aurora C18 column (Ion Optics) using a nanoElute. Peptides were separated on the column via a 30 min ACN gradient and eluted into a timsTOF SCP (Bruker). Data were acquired in dia-PASEF (data independent acquisition in parallel accumulation serial fragmentation) mode and analyzed with DIA-NNv1.8 in predicted library mode.

Preliminary data

Commercially available HeLa cell protein digests (Pierce) were used for gradient versus peptide load assessments and analyzed with 15, 30, and 60 min acetonitrile gradient lengths. The 30 min gradient performed best for peptide loads up to 5 ng, the 60 min gradient was more beneficial for loading > 5ng.

The CellenONE platform sorted and prepared HEK and HeLa cells were run with the 30 min gradient and data acquired in dia-PASEF mode on a timsTOF SCP. Data processing in DIA-NN using an ion mobility and retention time predicted library of human protein sequences without match between runs identified for HEK cells about 800 protein groups and 3000 peptide sequences reproducibly from a single HEK cell going up to 3,700 protein groups and 22,000 sequences from 20 cells. For HeLa cells, about 1,100 protein groups with 5000 peptide sequences from a single cell and 3,500 with 24,500 peptide sequences from 20 cells were reproducibly identified by this workflow.

Quantitative comparison using principal component analysis clearly separated single HEK cells from single HeLa cells while single cells from on cell type clustered closely together. Several secretoglobulins as well as desmosomal proteins such as desmoplakin and desmoglein 1 were either present at a higher abundance or were exclusively found in HEK cells. These proteins are typical for early embryonal kidney development.

ABSOLUTE QUANTITATIVE PROTEOMICS OF CHROMOPHOBE RENAL CELL AND RENAL ONCOCYTOMA

Eduardo Galaricha[1], Susana Jorge[1,2], José L. Capelo[1,2], William LaFramboise[3], Swati Satturwar[3], Dimitrios Korentzelos[3], Sheldon Bastacky[3], Gabriela Quiroga-Garza[3], Rajiv Dhir[3], Jacek R. Wiśniewski[4], Carlos Lodeiro[1,2] and Hugo M. Santos[1,2,3*]

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Abstract

Oncocytic renal neoplasms are characterized by overlapping histologic, immunohistochemical, and molecular features that challenge accurate diagnosis [1]. The subset of renal oncocytic neoplasms includes the benign renal oncocytoma and the malignant chromophobe renal cell carcinoma [1, 2]. In this work, we demonstrate the total protein approach based on high-resolution label-free mass spectrometry as a tool to improve the accuracy of oncocytic renal neoplasms. MS-data was validated using semi-quantitative immunohistochemistry with 128 samples assessed on tissue micro-arrays. Our approach has identified and validated LAMP1 and HK1 as efficient markers of chromophobe renal cell carcinoma and renal oncocytoma, respectively [2].

Acknowledgments

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II.15

A new approach for easy and reliable Bladder Cancer Diagnostic and Monitoring

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Biomarker discovery as a tool for diagnostic and prognostic of diseases has proven to have many shortcomings. The vast majority of recently discovered biomarkers for disease have very limited diagnostic and prognostic value. Many of these biomarkers stem from physiological conditions brought on by the disease, but cannot be directly linked to the disease itself. Another contributing factor to these shortcomings is patient phenotype and the role it plays in biomarker response. However, modern mass spectrometry has opened a world of new possibilities to use the evolution of the patient's proteome to evaluate the disease progression. High-resolution mass spectrometry allows for the monitoring of the levels of thousands of proteins, instead of just a few biomarkers, and can provide insight into the roles proteins play in the biochemical pathways they are involved in. Thus, it becomes easy to evaluate which biochemical pathways the disease is affecting, as well as treatment response, providing useful insight into therapy adjustment. This concept was applied to a pool of patients with different stages of bladder cancer, ranging from Ta, T1, and T2+, and it was shown that through this approach it is possible to both provide an accurate diagnosis of disease stage, while also providing valuable insights into patient outcome.

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Autoimmune Hepatitis and Primary Biliary Cholangitis: bottom-up analysis of the acidic insoluble fraction of salivary proteome

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Autoimmune Hepatitis (AIH) and Primary Biliary Cholangitis (PBC) are autoimmune liver diseases characterized by liver fibrosis, chronic inflammation, and metabolic changes. There are still critical diagnostic issues due to the invasiveness of diagnostic methods and the sharing of symptoms with many other liver diseases. Saliva represents a less invasive biofluid candidate for diagnostic and prognostic purposes since it contains both proteins from salivary glands and other sources such as cells lining the oral integuments and bloodstream [1].

A bottom-up proteomics approach was used to analyze the acidic insoluble fraction of saliva from 15 AIH patients, 15 PBC patients, and 15 age/sex matched healthy controls (HC) with the aim of identifying qualitative/quantitative changes of the salivary proteome. Based on our previous results obtained by a top-down approach on the acidic soluble fraction of saliva, most of the peptides/proteins with altered levels among the three groups were found in the molecular mass range under 30 kDa. Hence the samples of the acidic insoluble fraction of saliva were submitted to SDS-PAGE under reducing conditions, and only gel portions containing the lowest molecular weight proteins (</= 25 kDa) were subjected to tryptic digestion, and nano-HPLC-high-resolution-MS/MS analysis combined to label free quantitation. The software Proteome Discoverer v.2.2 (Thermo Fisher Scientific), MaxQuant v.2.0.3.1 (Max-Planck-Institute of Biochemistry) and PEAKS Studio Xpro (Bioinformatics Solutions Inc., Waterloo, Ontario, Canada) were used for proteins identification and quantitation. Statistical analysis was made with Perseus v.1.6.5.0 [2] and MetaboAnalyst 5.0. Statistically varied proteins (*p-value*</=0.05) between the three groups were submitted to online resources STRING v.11.5 [3] and Reactome Pathway Databases v.79 [4] for functional and pathways enrichment analyses.

2174 proteins were identified, 816 of them with high confidence. Group comparison revealed that 32 proteins are statistically dysregulated between AIH and HC, 45 between PBC and HC, and 48 between AIH and PBC. In addition, PLS-DA analysis evidenced Immunoglobulin heavy variable 3-72 (IGHV3-72), Polymeric immunoglobulin receptor (PIGR), Cystatin-SN (CST1), Cystatin-SA (CST2), Cystatin-S (CST4) and Protein S100A9 (S100A9) among the proteins most discriminating the three groups. Moreover, results from enrichment analysis highlights that proteins with altered levels are mainly involved in processes related to immune system, inflammation, and metabolic activity. This preliminary study, that correlates the salivary proteome with the pathological condition, constitutes the basis for a better possible use of saliva as a diagnostic fluid in these pathologies and for a more accurate discrimination of subjects based on AIH or PBC occurrence.

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BLOOD PROTEOMIC ANALYSIS OF THE EFFECT OF PHYSICAL EXERCISE IN OLDER ADULTS

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The prevalence of age-related diseases, such as dementia or frailty, has recently increased due to the rise in the population aging. Thus, it becomes essential to analyze possible preventive measures that act on the risk of mild cognitive impairment (MCI) as well as the improvement and preservation of functionality. Although there is no recommended strategies for treatment and prevention of MCI, the benefits from non-pharmacological intervention such as physical exercise have been suggested.

The aims of this project are to analyze the proteomic profile and ageing biomarkers in PBMCs and plasma samples of adults aged between 65 and 75 years old, analyzing the effect of a 5 months physical exercise program. The present work gather data from the INTERMAE project (Clinical Trial registration: NCT03923712).

The analyses were carried out by high-resolution liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based proteomics. PBMCs samples were processed using label-free approach using data-independent-acquisition (DIA) mode without using spectral a single-shot libraries (direct-DIA), whereas plasma samples were analysed with a library generated from the data-dependent acquisition (DDA) runs (DIA analysis). In total, 6295 proteins were identified in PBMCs while 347 proteins were found in plasma. The functional analysis will be crucial to discover not only the processes influenced by ageing but also the molecular mechanisms that could be affected by physical exercise.

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Exploring the role of secretome on somatic embryogenesis efficiency in *Olea europaea* L.

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Due to the maintenance of the genetic background of the donor plant and the single-cell origin of the somatic embryos, somatic embryogenesis (SE) is being used on several important agronomical species not only for clonal plant propagation, but also to assist plant breeding through genetic engineering protocols. The olive tree (*Olea europaea* subsp. *europaea*) presents a recalcitrant behaviour concerning SE when adult tissues are used as initial explants, thus, limiting its routine use. To overcome this SE recalcitrance response, the role of different factors was evaluated (type of initial explant, development and physiological stage of the explant, growth conditions, chemical composition of the culture media), but limited improvements were achieved so far. It is known that the release of organic bioactive molecules by the explants into the culture medium affects the SE response in several plant species, but it has never been studied in olive tree. Of those molecules, the extracellular proteins (known as secretome) were previously described as indispensable players in the differentiation and morphogenesis of the somatic cells. Therefore, an exploratory approach aiming to characterize the secretome of liquid embryogenic cultures of olive is presented.

Olive somatic embryogenic cultures were established using cotyledons and radicles taken from mature embryos of seeds collected from cv. 'Galega vulgar' trees. The liquid embryogenic cultures were established based on the protocol developed by our research group. The secretome was extracted from 100 ml of liquid embryogenic cultures using 10 kDa cutoff centrifugal devices, followed by TCA/acetone precipitation. The protein extract was digested with trypsin using S-Trap™Micro Spin columns and further analysed by LC-MS/MS using a Orbitrap Exploris™ 240 Mass Spectrometer.

A total of 1127 proteins were identified, of which a significant number is involved in plant response to stimuli and stress, and in metabolic processes. Heat-shock protein 70 (HSP70), previously associated with SE in different plant species, was identified among the characterized proteins. A detailed characterization and the functional analysis of the main pathways identified will be presented and discussed.

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A mass spectrometry-based proteomics approach for FFPE laser-capture microdissected renal tissue analysis focused on Monoclonal Gammopathy of Clinical Significance

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Monoclonal gammopathy of uncertain significance (MGUS) affects 3% of population over fifties and frequency increases with age. MGUS patients have an abnormal immunoglobulin binding protein in serum, known as monoclonal protein or protein M, and commonly, no treatment is required. However, some patients develop organ or tissue damage by unknown toxic mechanisms. The concept of MG of clinically significant (MGCS) has raised awareness of the serious diseases derived from protein M. The need for a rapid intervention to preserve organ function, even without criteria for haematological neoplasia, claims for early diagnosis (1). This proof-of-concept study seeks to help in clinical practice and to understand tissular injury mechanisms by establishing a proteomics approach to characterize molecular protein signatures of renal toxicity.

We adapted single cell proteomic workflows to achieve maximum proteome coverage for the quantitative profiling of renal samples in MG patients, starting from low protein amounts (3 to 6 glomeruli) extracted by laser-capture microdissection (LCM) from formalin fixed paraffin-embedded (FFPE) tissue.

Prior to tissue collection by LCM, samples were deparaffinized. Protein lysis and extraction was performed in 50%TFE-Tris buffer, avoiding detergents and protein digestion with Lys-C trypsin in 10% TFE-Tris buffer (2, 3). Samples were injected in an Evosep system using Whisper methods coupled to an Orbitrap Eclipse, exploring different acquisition MS2 methods with and without real time search options. Data analysis for protein ID and quantification was done with PD and MQ software and statistical packages.

Using minimal sample preparation workflows, and state-of-the-art data dependent acquisition (DDA) MS workflows to enhance sensitivity, we consistently analyzed very low input protein amounts (tissue areas of approximately 58.000 μm^2 to 420.000 μm^2 (10 µm thick sections with 22 to 167 ng of total initial protein) in 58 min single-run analyses. Our results highlight the potentiality of using LCM to isolate glomerular areas in FFPE renal biopsies for patient phenotyping using unbiased proteomics and prove the feasibility of analyzing large tissue cohorts in a robust, timely manner with the final goal of seeking for MGCS biomarkers.

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Exercise training modulates mitochondrial proteome plasticity in murine urothelial carcinoma-induced cardiac remodeling

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Exercise training has been shown to reduce cardiovascular complications in various diseases, but the effect in cancer-related cardiac dysfunction is poorly comprehended. To gain new insights into the potential therapeutic effect of exercise training on the cardiac remodeling in cancer set, we characterized the molecular pathways in the heart mitochondria of a mouse model of chemically-induced urothelial carcinoma that was subjected to 8 weeks of high-intensity treadmill exercise using mass spectrometry. The data showed that exercise prevented the left ventricular diastolic dysfunction, fibrosis, and structural changes observed in mice with tumour. At the mitochondrial level, exercise training counteracted the lower capacity for ATP production observed in the hearts of animals with urothelial carcinoma and induced the upregulation of fatty acid oxidation and the downregulation of the biological process "cardiac morphogenesis." Moreover, exercise training promoted the upregulation of the biological processes "ATP metabolism" and "Smad signal transduction," whose regulation seems to involve the activation of specific kinases such as Rad53 or SGK. Overall, our data support the recommendation of exercise training for the management of cancer-related cardiac dysfunction and highlight the contribution of specific kinases that could be seen as targets for the development of new pharmacological strategies.

Poster Session III

Application of diaPASEF proteomics in the discovery of new lung cancer biomarkers in sputum

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Sputum is a fluid that contains lung airway epithelium and whose proteins allow, by means of proteomic techniques, to determine possible biomarkers in lung cancer (LC). The aim of this study is to investigate whether the sputum proteome can be used as source of proteins with differential abundance in patients with and without LC. In this single-center case-control study, adults with LC (cases) and controls without LC were included. Protein extracts were obtained from sputum samples and subjected to digestion with trypsin. Peptide digests were analyzed by nano-liquid chromatography coupled to mass spectrometry (nLC-MS), using a hybrid Q-TOF spectrometer with ion mobility (Bruker TimsTOF Pro) and working in diaPASEF acquisition. The runs were processed with the directDIA workflow in Spectronaut software, and differential abundance was assessed with the limma test. For functional analysis, the result from differential expression analysis was further analyzed in the STRING platform, and feature selection was performed by sparse partial least squares - discriminant analysis (sPLS-DA). Significant differential abundance (fold-change>1.5 and p-value<0.01) was found in 33 proteins. Our results showed an enrichment in proteins related to inflammatory response, complement and coagulation cascade. The resulting sPLS-DA model separated between case and control groups with good sensitivity and specificity. In conclusion, according to our results, new generation proteomics of sputum samples could allow non-invasive diagnosis of LC in the future.

High-resolution MALDI-MSI workflow for proteomic analysis of zebrafish embryos

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Endocrine-disrupting chemicals (EDCs) are a relevant environmental threat as a result of their capacity to dysregulate the homeostasis of endogenous hormones. Zebrafish (Danio rerio) is a powerful model organism in ecotoxicology for studying EDCs, strikingly, toxicological data from zebrafish can be generalized to other vertebrates, including humans [1]. Also, it presents a huge variety of advantages concerning other common model organisms, such as its effortless manipulation or large offspring. Moreover, zebrafish embryos are considered an excellent alternative animal model, providing similar results as adult animals in toxicity studies including EDC assessment while submitted to less ethical restrictions [2].

Omic technologies have been widely used in environmental toxicology for a better understanding of the response of an organism to pollutants exposure, being especially useful to characterize their mode of action (MoA). Traditional omics are based on bulk methods, where individual or pools of whole tissues are analyzed, providing a useful average view of the molecular landscape. However, with this approach, the molecular information of individual cell types and the spatial organization cannot be achieved, which is extremely relevant when analyzing heterogeneous samples as zebrafish embryos. To overcome these challenges, breakthrough technologies have recently emerged to encompass single-cell and spatially resolved omics. For example, the development of powerful tools such as spatially resolved mass spectrometry technologies, including mass spectrometry imaging (MSI), allows incorporating spatial resolution into omics studies [3].

In this work, we developed and optimized a spatial proteomics workflow using an HR-MALDI-TOF with a lateral spatial resolution of 10 µm to be implemented in zebrafish embryo sections. Our results showed that a weakly basic medium (pH=8.5) is required for enhancing trypsin digestion and therefore, achieving good peptide profiles. An initial exploratory analysis using a pLSA tool has demonstrated the usefulness of the MSI approach to discriminate different regions of interest (ROIs) of the zebrafish embryos. Remarkably, the posterior clustering analysis allowed the association of these clusters with different parts of the zebrafish embryo (e.g., eye, brain, digestive system, tail), demonstrating the promising future of high-resolution spatial proteomics studies in zebrafish embryos. Further studies will be focused on integrating spatially resolved transcriptomic and lipidomic data to assess the toxicological effect of EDCs.

Keywords: Spatial proteomics, mass-spectrometry imaging, zebrafish embryos

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III.23

SUMO-ID: biotin-based identification of proximal SUMO-dependent interactors using mass spectrometry

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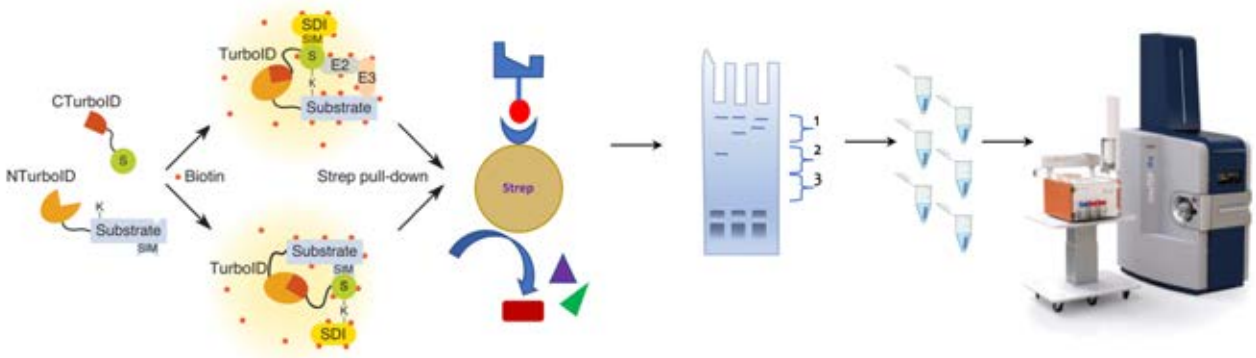
The fast dynamics and reversibility of posttranslational modifications by the ubiquitin family pose significant challenges for research. We have developed SUMO-ID, a technology that merges proximity biotinylation by TurboID and protein-fragment complementation to find SUMO-dependent interactors of proteins of interest. Using an optimized split-TurboID version, we show SUMO interaction-dependent labelling of proteins proximal to PML. SUMO-dependent interactors of PML are involved in transcription, DNA damage, stress response and SUMO modification and are highly enriched in SUMO Interacting Motifs, but may only represent a subset of the total PML proximal proteome. We show that the method may be used for other ubiquitin-like proteins, identifying SUMO1-, SUMO2-, and UB-dependent interactors of the tumor suppressor p53. Biotinylated proteins are captured on neutravidin resin, which is subjected to stringent washes to reduce background contaminants, and then eluted in Laemmli buffer with heating. Polyacrylamide gel electrophoresis and SYPRO-Ruby staining allow gel slices to be visualized and excised, while avoiding neutravidin contaminants. In-gel trypsin digests are then put into a proteomics pipeline consisting of an EVOSEP ONE (nLC) coupled on-line to a timsTOF Pro (MS/MS). Data analysis is performed using MaxQuant and Perseus software packages. Taken together, SUMO-ID is a powerful technique that allows to study the consequences of SUMO-dependent interactions, and shall help to further unravel the complexity of the ubiquitin code.



Proteomics analysis of single human oocytes using magnetic beads based digestion and ultra-sensitive MS acquisition

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Mammalian oocytes are exceptional cells in that they are extremely long-lived and start the meiotic program during fetal development, their growth is kept arrested for a long period of time, and become recruited from the resting to the growing pool for final maturation and fertilization [1]. Although it might be of paramount importance for understanding human reproduction, the characterization of gene translation into functional protein products at different stages of oocyte growth and maturation remains poorly understood.

Taking advantage of novel sample preparation (SP3) and sensitive acquisition methods in a timsTOF Pro powered with PASEF from Bruker, we have been able to successfully analyze protein starting from single oocytes. This approach was applied to the analysis of the proteome of oocytes at different maturation stages (Germ vesicles, Metaphase I and Metaphase II, n=4 per group). A total of 1038 different proteins were identified, of which 640 proteins were identified with at least two different peptides in at least 50% of one of the groups. The analysis of this subset of proteins revealed protein abundance changes that are coherent with differences already described in the literature, such as the downregulation of YBOX2 or the increase in WEE2 and PCNA upon oocyte maturation, among others.

This preliminary characterization opens the door to novel approaches to come, such as the analysis of differential oocyte protein abundance in the context of pregnancy disorders.

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DIFFERENTIAL PROTEIN EXPRESSION ANALYSIS OF SYSTEMIC LUPUS ERYTHEMATOSUS TO LUPUS NEPHRITIS EVOLUTION BY LABEL FREE LC-MS

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Systemic lupus erythematosus (SLE) also called only “Lupus” is a rare autoimmune disease in which the body’s immune system attacks healthy tissue in many parts of the body, with symptoms varying from mild (hair loss, chest pain, fever…) to severe (several organ inflammation diseases). In severe forms, SLE evolve into Lupus Nephritis (LN) in 30 to 50% of cases, which is the inflammation of the kidneys (a form of glomerulonephritis) with a bad overall prognosis, with five-year mortality rate of 5 – 25% in those severe cases with progression to kidney failure (10 to 30% of LN cases). Once diagnosed, is common that patients get into advanced LN stage, making difficult the treatment and disease control. Thus, there is an urgent need of specific, sensitive and non-invasive methods of diagnosis and prognosis not only for the differentiation between early and advanced LN stages, but also for SLE to LN evolution.

Nowadays, proteomics plays a fundamental role not only in cellular and molecular biology research but also in clinic diagnosis and prognosis. Besides, urine has been used extensively in several disease studies as a non-invasive, easy to obtain, and stable sample. This work will search for protein differences in urine samples from a cohort of SLE patients versus LN patients, in several disease stages, with the aim of study the proteins involved in the crossing from SLE to LN. This proteomic analysis was performed following a label free quantification method. Panels of several protein candidates have been obtained for different stages. Eventually, this information could be used to obtain panels of protein markers focused on clinical diagnosis, prognosis and disease treatment monitoring.

Keywords: Lupus, Nephritis, LC-MS, Label-free quantitation, proteomics

Comparison of nitrate assimilation in three marine *Synechococcus* strains

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The marine picocyanobacteria *Prochlorococcus* and *Synechococcus* are the most abundant photosynthetic organisms on Earth, play an important role in the oceanic regeneration of nutrients and are responsible for a significant part of the global primary production [1]. They have demonstrated a high capability to adapt to environments with different availability of nutrients, light and temperature in the oceanic ecosystems [2].
These microorganisms require nitrogen (N) as an essential nutrient for growth, but the availability of this element is a limiting factor in the oceans [3,4]. N can be found in the oceans in the form of ammonium, urea, nitrite, nitrate or amino acids. Nitrate assimilation is of particular interest since it is an abundant N species in oceanic environments, although it is a costly source for the cell to metabolize as it is completely oxidized. *Synechococcus* thrives in mesotrophic and moderately oligotrophic waters, where almost all strains are able to utilize both oxidized and reduced forms of nitrogen.
In this work we present a detailed study, including proteomic analyses and gene expression by RT-qPCR of genes of interest, of the response of different *Synechococcus* strains to the availability of several N species. Three *Synechococcus* strains (WH7803, WH8102 and BL107), inhabiting different zones of the ocean, were grown under four different conditions: standard ammonium concentration, no added nitrogen source, micromolar nitrate concentration or nanomolar nitrate concentration as sole nitrogen source. Our study shows that *Synechococcus* sp. WH7803 has a different response to nanomolar nitrate concentrations than to the absence of nitrogen or the presence of standard ammonium or nitrate concentrations. Comparative studies with the WH8102 and BL107 strains will be shown in this communication.

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Discovery of protein biomarkers for the diagnosis of equine metabolic syndrome

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Equine metabolic syndrome (EMS) is a complex disorder of which pathogenesis is poorly understood. This syndrome is characterized by the presence of three factors: obesity, insulin resistance and episodes of laminitis [1]. The most common and painful clinical consequence of this disease is laminitis, which causes damage to the horse's hoof even before the symptoms of the syndrome are noticeable. Acute laminitis is often a cause of extreme suffering, and its chronic effect can lead to euthanasia of the animal [2]. Early diagnosis of metabolic syndrome is essential to take preventive measures to avoid the development of laminitis [3]. Currently, there isn´t specific diagnostic test for equine metabolic syndrome [4]. In view of this, it is essential to develop an effective and sensitive test that allows the veterinary clinician prompt diagnosis. Quantitative proteomics has proven to be a valuable tool for protein identification, characterization and quantification [5].

One of the areas in which proteomics developments have been more acknowledged is biomarker discovery and quantification. The comparison of the protein pattern of a pathological versus a healthy state offers the opportunity to discover protein-based biomarkers for early diagnosis. Moreover, identifying proteome profiles represents an important step towards disease characterization, since differentially expressed proteins may provide information about the pathophysiology of the disease. The aim of our project is to identify a panel of specific biomarkers for the EMS in plasma that will serve to develop a specific diagnostic method to guide veterinary clinicians to diagnose this disease in the animal before it manifests irreversible damage.

In the present communication, we will show the results obtained so far on the optimization of a depletion strategy in plasma and a pilot study using quantitative mass spectrometry-based proteomics to discover and quantify protein biomarkers for the diagnosis of EMS.

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Identification of differential proteomic profiles in the synovial tissue of patients with rheumatoid arthritis and psoriatic arthritis

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The differential diagnosis of Rheumatoid Arthritis (RA) and Psoriatic arthritis (PsA) is often difficult due to the similarity of symptoms and the unavailability of reliable clinical biomarkers. Molecular alterations have been suggested to contribute to the pathophysiological processes in the knee joint, and it is known that chronic inflammation induces significant changes in the synovial tissue (ST) and synovial fluid (SF) lipidome and proteome. Previous work has demonstrated the presence of specific lipid markers in ST and SF. In the present study we aimed to evaluate whether differences in ST proteomic profiles could also support the diagnosis of these diseases. To this end, a quantitative proteomic analysis was carried out on FFPE ST from RA (n=13), PsA (n=13) and controls (n=8) by nLC-MS/MS analysis using a TimsTOF Pro system (Bruker). Statistical analyses were performed using GraphPad Prism, Metaboanalyst and LFQ Analyst software.

Around 2,500 distinct proteins were identified in the ST, including several that are related with lipid metabolism. Near 300 of them showed altered abundance in the pathological tissues compared to healthy controls (FDR 0.01%, Fig 1A), being the small subset increased in controls mainly extracellular matrix proteins. The comparison between RA and PsA ST led to the identification of a panel of 36 proteins discriminating the two tissues with high statistical significance (p-value <0.01). In this comparison, all proteins except two appeared increased in RA (Fig 1B). A discriminant analysis shows the usefulness of this protein panel to differentiate the two diseases (Fig. 1C).

In conclusion, our study shows distinct proteomic profiles between RA and PsA synovial tissue and reports potential clinically useful protein markers for the differential diagnosis of these diseases.

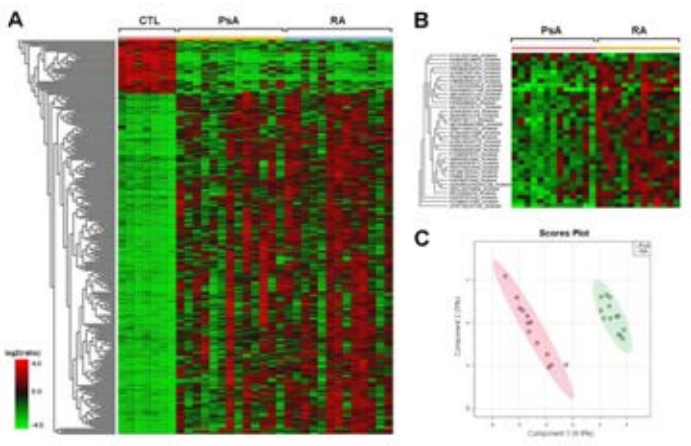

Figure 1. Results from the proteomic analysis carried out on synovial tissues. A) Heatmap showing the differential protein profiles between synovial tissues (PsA and RA) and healthy controls (CTL), at FDR 0.01. B) Characteristic protein panel discriminating PsA and RA tissues (p-value < 0.01). C) Discriminant analysis performed using this protein panel.

An affordable proteomic perspective on the search of sustainable plant-based proteins with technological applications in food industry

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Abstract

There is an increasing interest to shift consumer habits toward plant-based diets considering health and environmental aspects. Thus, scientific actions are urged to address the development of innovative high-protein meal formulations from common plants as healthy and sustainable protein alternatives. This research aims to provide a readily accessible analytical strategy enabling the proteomic characterization of plant flours from legumes (chickpea, lentil, red lentil and white bean), oat and pseudo-cereals (amaranth and quinoa) through an affordable LC-MS-based proteomic pipeline for subsequent comparison with their techno-functional properties.

Detailed protein profiles of flours assayed were achieved by gel-based enrichment/fractionation with quantitative image analysis hyphenated to a tandem MS/MS qualitative research featured by a three-dimensional ion-trap. Results from major proteins were correlated with techno-functional properties of raw materials studied. Legumins, vicilins, convicilins and provicilins mainly characterized the legume flours proteome and could explain their interesting emulsifying and foaming capacities. In contrast, the extremely high content of phaseolin and to a lesser extent of agglutinins in white bean sample may be the answer of its non-gelling behavior and scarce foaming stability. Furthermore, although quinoa, oat and amaranth flours were rich in globulins, their techno-functional properties related to an enhanced water and oil holding capacity seemed to be the result of the association between protein and fiber contents. Other minor proteins were also detected in samples such as Len c 1.0101/1.0102, alpha-amylase inhibitors, enolase, peroxygenase and avenin that would deserve further studies prior to know their relevance in food processing.

Results achieved in this study can create new insights on the use of plant-based proteins to produce innovative, healthy, affordable and sustainable food products.

Protein interactions : how to decipher them?

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Protein interactions are essential in all living cells to ensure crucial functions and thus to understand better these processes, it is important to identify which protein interacts with which others. Co-fractionation-based methods combined to mass spectrometry and Edman sequencing are complementary to decrypt these interactions. In the green alga *Chlamydomonas reinhardtii (Cr)*, we characterized *in vitro* complexes: i) the Glyceraldehyde-3 phosphate dehydrogenase (GAPDH) with the CP12, by MALDI-ToF peptide mass fingerprint [1] and ii) the GAPDH-Adenylate Kinase 3 complex, by LC-MSMS [2]. We also proved that GAPDH and Ferredoxin-NADP reductase, from the diatom *Asterionella formosa,* were interacting [3]. Interaction between a diheme cytochrome c4 and a cupredoxin, was deciphered, demonstrating the intermolecular electron transfer in the respiratory chain from an acidophilic bacterium [4]. Protein oligomerization is important to reach an active conformation: the iota-carbonic anhydrase in the diatom *Thalassiosira pseudonana* (Thaps*)*, is tetrameric (SEC and native ESI- Q-ToF-MS) [5]. CP12 from Thaps is a dimer, bringing new structural and functional insights in IDP family [6]. APEX2, was used on *E. coli*: the assembly of the type VI secretion system, a multiprotein weapon used by bacteria to destroy competitor cells, was detailed by this proteomic approach, highlighting spatially interacting partners into the nano-crossbow-like machinery [7]. *In vivo* global approaches using quantitative proteomics and mutant strains: a transgenic line of the marine diatom *Phaeodactylum tricornutum* produced to accumulate ppGpp [8], ii) a *Cr* strain deleted on CP12 [9]), bring dozens to a hundred proteins affected in the biological pathway targeted. Subsequently, other biochemical/biophysical methods will be required to confirm further these proteomics data.

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Poster Session IV

TMT-based quantitative proteomics analysis reveals differentially expressed proteins between different sources of hMSCs

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Human mesenchymal stem cells (MSCs) are currently the leading candidate for cell-based therapeutics. While the use of MSCs in transplantation therapies is widely expanding, still, there is a lot of scope for better understanding of the mechanisms underlying their effects. One of the main sources used is bone marrow (BM) autograft, which in the case of bone repairs involves additional surgery and potential complications. In this preliminary study, we compared BM-MSCs with others MSCs sources that can be more easily collected: Wharton-Jelly's (WJ)-MSCs from umbilical cord, available at birth, and dental pulp (DP) MSCs, which can be harvested from wisdom teeth or deciduous teeth extractions. Here, we conducted a tandem mass tags (TMT)-based quantitative proteomic analysis from WJ, BM and DP-MSCs. Our results show that all samples express MSC markers, with a difference in the expression of CD44, CD166 and CD105 depending on the MSC sources. Most of the differences of expression were observed with protein implied in the regulation of biological processes (190 proteins) and/or metabolic processes (145 proteins) and/or response to stimulus (145 proteins). DP-MSCs expressed significantly more proteins involved in the extracellular matrix (ECM) than BM- and WJ-MSCs. MSC-WJ express more than other sources, proteins involved in cell differentiation, defense response: inflammatory and immune. These results suggest that MSCs from different sources appear to have different potentials and secretion patterns, which could influence their therapeutic action.

MS-based quantitative proteomic analysis of serum-purified exosomes for the identification of pre-eclampsia-associated biomarkers

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Preeclampsia is one of the leading causes of fetal and maternal morbidity and mortality, affecting 2-7 % of pregnancies. Identification of early diagnostic biomarkers, before the appearance of the first clinical symptoms, would facilitate the development of new strategies for preeclampsia prediction that would allow the early intervention. Serum-purified exosomes emerge as a promising source of protein biomarkers that circumvents some of the inherent challenges of proteome wide analysis of plasma/serum. Besides, purified exosomes have the added interest of being communication vehicles between cells and tissues both in physiological and pathological processes.

We quantitatively compared the protein composition of exosomes purified from three different collections of control and pre-eclamptic serum samples, obtained at the end of the second trimester of pregnancy and at delivery. Exosomes were purified using a molecular size exclusion-based isolation procedure. We performed shotgun label-free proteomics to investigate differential protein expression, and differentially regulated proteins were then validated by targeted proteomics.

The efficiency of the exosome isolation method was demonstrated by the identification of a significant number of protein markers of exosome origin. We also identified pregnancy-specific proteins in line with previous studies suggesting that a high percentage of purified exosomes derived from pregnancy-related tissues. Shotgun quantitative proteomic analysis determined 10, 114 and 98 differentially regulated proteins, respectively, in the three-independent serum-isolated exosome collections. A high degree of agreement was detected between the three sets of differential proteins. Functional analysis of the differentially expressed proteins showed that they were involved in biological processes closely related to pre-eclampsia, such as angiogenesis, inflammation, or cell migration. Likewise, differential abundance of 66 exosomal proteins was validated by targeted proteomics (S/MRM). Finally, functional experiments performed on an *in vitro* cell-culture model demonstrated that the addition of purified exosomes from control and preeclamptic serum samples, induced a differential effect on the qualitative and quantitative composition of the targeted cell proteome.

The identification and validation of exosomal proteins differentially expressed in serum of pregnant women highlighted liquid biopsy as a promising source for early detection of pre-eclampsia.

THE SERUM OF ASYMPTOMATIC COVID-19 INDIVIDUALS AFFECTS CIRCULATING ANGIOGENIC CELLS FUNCTIONS. A PROTEOMIC APROACH.

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To date, more than six million people has died from Coronavirus-19 (COVID-19) disease since the pandemic was declared in March, 2020. The virus responsible, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), compromises the cardiovascular system causing vascular damage and thromboembolic events in critical COVID-19 patients, deriving in many related deaths and long-hauler symptoms. Understanding how these processes are triggered as well as the potential long-term sequelae, even in asymptomatic individuals, becomes essential.

Herein, we have evaluated, by application of a proteomics-based quantitative approach, the effect of serum from COVID-19 asymptomatic individuals over circulating angiogenic cells (CACs). Since CACs are thought to actively participate in vascular repair, we considered relevant to know the effects of SARS-CoV2 infection on CACs.

Thus, healthy CACs were incubated *ex-vivo* with the serum of either COVID-19 negative (PCR-/IgG-, n:8) or COVID-19 positive asymptomatic donors, at different infective stages: PCR+/ IgG- (n:8) and PCR-/IgG+ (n:8). Then, cell lysate after 24 hours incubation were prepared for a label free quantitative (LFQ) approach and analysis by TIMS-TOF Pro instrument (Bruker Daltonics) operated in data dependent acquisition (DDA) mode. Raw files were processed with MaxQuant and then loaded in Perseus for further statistical analysis.

Proteomics-based quantitative approach reveled 1438 proteins identified and allowed differential expression patterns (up- and down-regulated proteins depending on the conditions tested). Machine learning algorithms confirmed the discriminating potential of the proteins differentially expressed between the three groups. Besides, functional classification reported that proteins differentially expressed correlated with endothelial dysfunction and inflammatory response after viral infection in CACs, but also with coagulation process and leukocyte extravasation.

In conclusion, the incubation *ex vivo* of CACs with the serum of COVID-19 asymptomatic individuals affects CACs functionality, resembling endothelial dysfunction. Moreover, several proteins such as TLR2, MNDA or HSPA5 stood out as significantly discriminating markers in CACs in response to PCR or IgG+ serums.

Proteomics Identification of Novel Amyloid-β Plaques Interactors Associated to Alzheimer's Disease.

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Introduction and objectives

Amyloid-β plaques, caused by the extracellular deposition of amyloid-β peptides, are one of the main hallmarks of Alzheimer's disease (AD). Thus, the identification of Amyloid-β peptides interactors should allow a better understanding of the disease and of the pathological changes underlaying its development. We here aimed to identify by quantitative proteomics analysis novel protein interactors of Amyloid-β peptides by using a label free approach, S-Trap spin columns and brain tissue protein extracts of the left prefrontal cortex of AD patients and healthy individuals as controls.

Methods

For the detection of Amyloid-β peptide interacting proteins, biotinylated-β-Amyloid and scrambled-β-Amyloid peptides were used for pulling down protein interactors with Streptavidin Sepharose Magnetic beads incubated with protein extracts from the left prefrontal cortex brain tissue samples of AD patients at Braak V and VI and healthy individuals as controls. Interacting proteins were Trypsin digested onto S-Trap spin columns and analyzed with a Q Exactive. Three independent replicates were analyzed to identify and quantify interacting proteins by label free.

Result and discussion

Data analysis of the label free approach of three control, three AD Braak V, and three Braak VI independent replicates with MaxQuant revealed a total of 2211 proteins identified and quantified. Perseus data analysis allowed the identification of 973 and 1377 interactors dysregulated with a fold change ≥ 1.5 or ≤ 0.67 in AD patients at Braak V and VI, respectively, in comparison to controls. Importantly, some proteins previously associated to Amyloid-β plaques were identified among the interacting proteins, highlighting the accuracy of the approach. Taking together AD patients samples in comparison to control samples allowed us to identify and quantify 1115 proteins interacting with scrambled and Amyloid-β peptides. Of them, 426 interactors were specifically observed in common in Braak V and VI AD patients, which would be of further interest for the study of the disease.

Conclusion

Novel proteins interacting with Amyloid-β peptides were here identified by using label free approaches, S-Trap spin columns for digestion and pull-down analysis of Amyloid-β biotinylated peptides, which might help identifying novel proteins associated to AD pathology, which could be key in AD development, and should be further analyzed functionally.

Keywords: Alzheimer's disease, Proteomics, Immunoprecipitation, Label free, Amyloid-β plaques.

LIVING DONOR LIVER TRANSPLANT. UNDERSTANDING LIVER REGENERATION

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The liver has a unique regenerative capacity. Upon partial hepatectomy (PH), the liver undergoes a complex process that involves a finely tuned network of molecular events and interaction of different cell types to ensure efficient proliferation until restoration of liver size and function **(1)**. In some patients, however, liver regeneration (LR) is insufficient. A better understanding of the mechanisms involved in LR could identify new therapeutic targets to improve postoperative organ function **(2)**. In this study, we have analyzed the serum proteome temporal dynamics of seven healthy donors of living donor liver transplantation (LDTD) to identify blood biomarkers of an efficient LR.

Forty-eight serum samples from LDLT healthy donors at different time points after surgery were included in a SureQuant analysis **(3)**, which was performed in an EASYnLC 1200 coupled to an Orbitrap Exploris 480 MS. In all cases above 500 peptides were identified and quantified using Skyline-daily program, of which 97 were statistically differential (q value< 0.1). The regulation of these 97 proteins occurred mainly during the first 72 h, which parallels the timing of the early regeneration phase. Functional interpretation using STRING database and Ingenuity pathways Analyses (IPA) showed changes mainly in the coagulation and complement system, insulin signaling pathway**,** innate immune response, antioxidant response, mitochondrial activity and hippo signaling. In order to validate these altered functions in liver tissue, we have used a PH mouse model, focusing on the starting regeneration phase (1H, 3H and 9H after PH). We deepen into the proteome through different biochemical techniques as liquid chromatography-tandem mass spectrometry (LC-MS/MS), western-blot and real-time PCR. We demonstrated that insulin signaling, oxidative phosphorylation, FXR-FGF15/19 pathway**,** one-carbon metabolism and hippo signaling were the key canonical pathways involved in the regulation on the starting regeneration phase.

Thus, SureQuant targeted approach allowed the follow up of human liver regeneration through the analysis of the serum proteome and revealed the main changes that occur during the early regeneration phase. The future goal is to develop a statistical model to predict the outcome of LDTD donors and patients.

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Seroprevalence of SARS-CoV-2 antibodies in healthcare workers and pediatric patients using a Luminex® assay

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SARS-CoV-2 infection, causing the highly infectious disease COVID-19, has become a global health problem since the beginning of the pandemic until nowadays with the emergence of new variants, being a challenge for the scientific community. One of the most affected sectors has been healthcare workers (HCW) as they are on the front line of this virus. Children have also been a part of the affected population, most of them suffering the infection as a mild disease, although some children have been reported to have developed Pediatric Inflammatory Multisystem Syndrome (PIMS). In this study, we recruited 190 adults (HCW and their cohabitants) and 57 children, of which 12 developed PIMS. Using the Luminex® technique, IgG, IgA and IgM levels were analyzed for different SARS-CoV-2 analytes as well as for the RBD of alpha, beta, gamma and delta variants of concern (VOC) in order to know the seroprevalence among the different groups of participants, obtaining that seropositivity rates were higher in children than in adults with the following results (children *vs* adults): IgG (49.1% *vs* 11%), IgA (45.6% *vs* 5.8%) and IgM (35.1% *vs* 7.3%). We also compared this outcome with COVID-19 ELISA IgM/IgA (Vircell, S.L.), finding that a high number of false positives was obtained compared to a more sensitive technique such as Luminex®. In addition, a correlation study between wild-type RBD and VOCs RBD for each isotype was performed, obtaining that Alpha RBD had the highest correlation index for all isotypes, while those with the lowest correlation index were Delta RBD for IgG, Gamma RBD for IgM and Beta RBD for IgA.

Upgrading X!TandemPipeline and MassChroQ for fast and accurate timsTOF native raw data support for quantitative proteomics.

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X!TandemPipeline (Langella et al. 2017) and MassChroQ (Valot et al. 2011) are two free and open source software programs designed to perform protein inference and peptide quantification from extracted ion currents (XIC), respectively. X!TandemPipeline also performs database searches using the renowned X!Tandem engine (Craig et al. 2004). Originally written in Java, we have completely rewritten X!TandemPipeline in portable C++17 and associated it to MassChroQ into a single software which now features peptide identification, protein inference, and area under the curve XIC-based quantification. Additionnally, thanks to the technical specifications provided by Bruker, we implemented support for the timsTOF raw data format. This original software code now allows X!Tandem to perform database searches right on optimized data brokered to it by our reader of native timsTOF binary data. With a total control of the data processing, we were able to tightly optimize our software to ensure very fast access to the binary data. As a result, the current version identifies roughly the same amount of peptides as when using the X!Tandem engine on MGF data provided by the Bruker Data analysis tool but performs much faster, reducing processing time from 55 to 12 minutes on a typical quality control HeLa sample. Furthermore, it provides real time MS/MS peptide annotation and performs extremely fast ion current extractions and XIC chromatogram visualizations (typically, in less than five seconds for a 2 hour PASEF run). Using a common HeLa data set published by Meier et al. (2018, PXD010012), we demonstrate that our software identifies and quantifies significantly more proteins than MaxQuant and MSFragger (5783 vs 4977 and 3526, respectively) with similar median coefficient of variation (0.059 vs 0.049 and 0.070, respectively). Comparable results have been found using data set PXD014777 (Prianichnikov et al., 2020). Overall, our software always performs faster than all the other software offerings, while producing similar or better results.

The secretome of a fuel-ethanol *Saccharomyces cerevisiae* yeast starter during alcoholic fermentation – a preliminary study

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Microbial contamination of alcoholic fermentation processes such as winemaking and fuel-ethanol production, is a serious problem for the industry since it may render the product unacceptable and lead to large economic losses. In previous works, we demonstrated that several *Saccharomyces cerevisiae* strains, including the fuel-ethanol starter strain Ethanol-Red, secrete antimicrobial peptides (AMPs) derived from the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase that are active against wine and fuel-ethanol microbial contaminants [1-3]. In this work, we aim to get a more comprehensive insight into the entire secretome of this yeast strain during fermentation, which could improve our understanding of how yeasts survive and interact with other species during mixed-culture alcoholic fermentations. To this end, we are studying the proteome profile of the extracellular medium of *S. cerevisiae* Ethanol-Red strain at different timepoints of fermentation (0h, 12h, 24h, 48h, 72h, 144h, and 192h), through 1D and 2D electrophoresis. Cell-free supernatants were concentrated using ultrafiltration devices and cleaned up by precipitation with TCA/acetone. Protein extracts were first analyzed by SDS-PAGE (12.5%; 16 cm) and then by 2DE using IPG strips with a 4-7 pH gradient (7 cm) in the 1st dimension, and polyacrylamide gradient mini-gels (4-12%; 7 cm) from NuPAGE, in the 2nd dimension. Gels were silver-stained. The visual analysis of 1D and 2D maps show that one can perceive protein release to the extracellular medium after 24h of fermentation but the total number of protein bands or spots observed in the 1D and 2 gels is limited. No significant changes are observed afterward. Compared to proteomic profiles reported in the literature for S. *cerevisiae* cell surface, acid phosphatases are good candidates for the high molecular mass proteins observed in SDS-PAGE gels [4]; these proteins have an abnormal migration in IPG strips possibly due to a high glycosylation pattern. Protein identification using peptide mass fingerprinting is underway.

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Deciphering the complexity of extracellular vesicles (EVs) mediated vascular calcification using a proteomic approach

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Vascular calcification (VC) is a strong predictor of cardiovascular risk, particularly in chronic kidney disease (CKD) patients, associated with increased vascular stiffness, pulse pressure, left ventricular hypertrophy and atherosclerotic plaque burden [1]. VC is currently accepted as a highly controlled multifactorial process, where the release of extracellular vesicles (EVs) with calcification capacity play an essential role in mediating cell-induced matrix mineralization [2]. Still, questions considering mechanisms of EVs deposition in the extracellular space, and its relation with vesicle origin, loading, calcifying capacity and role in intercellular communication remain elusive. In this work we established a proteomic approach to characterize extracellular matrix (ECM)-deposited and cell media (CM) released EVs, from an *in vitro* model of VC consisting of primary vascular smooth muscle cells (VSMCs), bringing new knowledge into their specific characteristics and cargo. EVs deposited in the ECM and released into the cell culture media (CM) from VSMCs cultured in control (CTR) and calcifying (MM) conditions, were isolated by differential ultracentrifugation at 30.000 xg (30K) and 100.000 xg (100K). Characterization of isolated 30K and 100K ECM EVs and CM EVs from CTR and MM VSMCs, was performed by transmission electron microscopy (TEM) and dynamic light scatering (DLS), showing different morphological and sized populations between 30 and 100K. Characterization of the different EV populations was performed by labelfree mass spectrometry-based proteome analysis, leading to the quantification of 2,057 proteins. Several EV markers, such as CD63, CD81, CD9, Flotilin 1, annexins A1, A2, A6, A5, A7, A11, were identified in all sample groups. Quantitative comparative analysis clearly showed different protein cargoes between EV populations isolated at different centrifugation forces and between ECM and CM samples. Differentially expressed proteins between CTR and MM EVs were mostly identified in the 30K ECM EVs, with 72 proteins found upregulated in 30K ECM CTR EVs. These results highlight the complexity of EV-mediated VC and reinforce the need for further research focusing on mechanisms governing EVs release, accumulation, functionality and intercellular communication.

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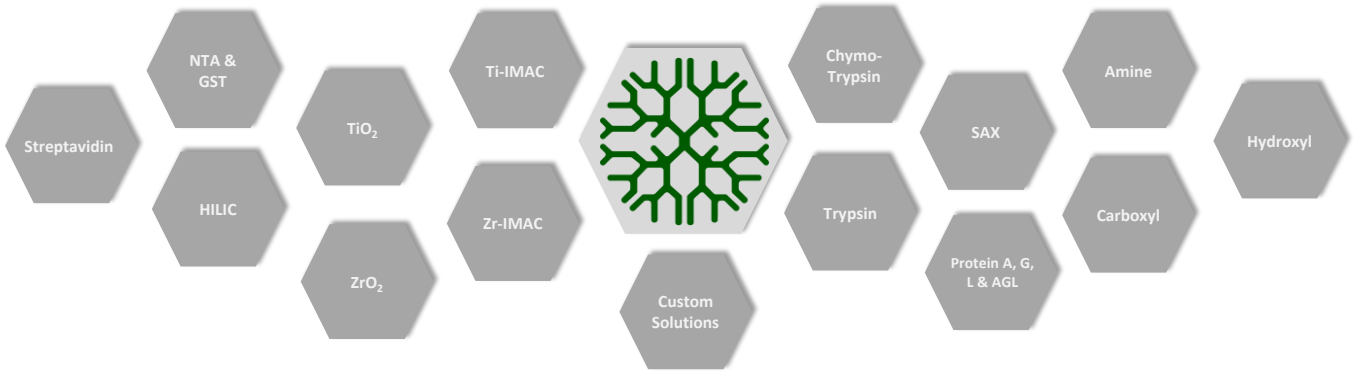

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